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A NEW TREMATODE PARASITE OF *ACIPENSER STURIO* L. (ROYAL STURGEON), WITH A DESCRIPTION OF THE GENUS *DIHEMISTEPHANUS* LSS.

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(With Plate XXXIX and 10 Text-figures.)

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INTRODUCTION.

ON November 14th, 1929, the writer examined a Royal Sturgeon, *Acipenser sturio* L., for trematode parasites. Up to the time of writing, this is the largest Sturgeon landed at Swansea, measuring 11 ft. long, 4 ft. 9 in. girth, and weighing 565 lb.; it was captured west of Lundy in the Bristol Channel by the steam trawler *Conway Castle* of the Consolidated Fisheries Ltd., Swansea.

Two living specimens of *Derogenes varicus* (Mueller) were found in the oesophagus and a new species of trematode herein described was located in the intestine.

During life the parasite is capable of considerable elongation and contraction particularly in the pre-acetabular region; specimens appear rust-coloured, due probably to the presence of numerous eggs in the post-acetabular region. Three of the four living specimens were found clinging to the mucous membrane lining the small intestine, the fourth being detached and embedded in the congealed chyme in this region.

After killing and fixing, the worms measured 3·5–6·2 mm. long with a maximum breadth of 0·49–0·69 mm.; two specimens were mounted *in toto* and two serially sectioned transversely.

EXTERNAL APPEARANCE.

The **body** is long and narrow (Pl. XXXIX, fig. 1), having a ratio of 6 : 1 to 9 : 1 semi-contracted; the breadth of the body gradually increases towards the posterior end which is bluntly rounded. **Around the oral sucker** (Pl. XXXIX,

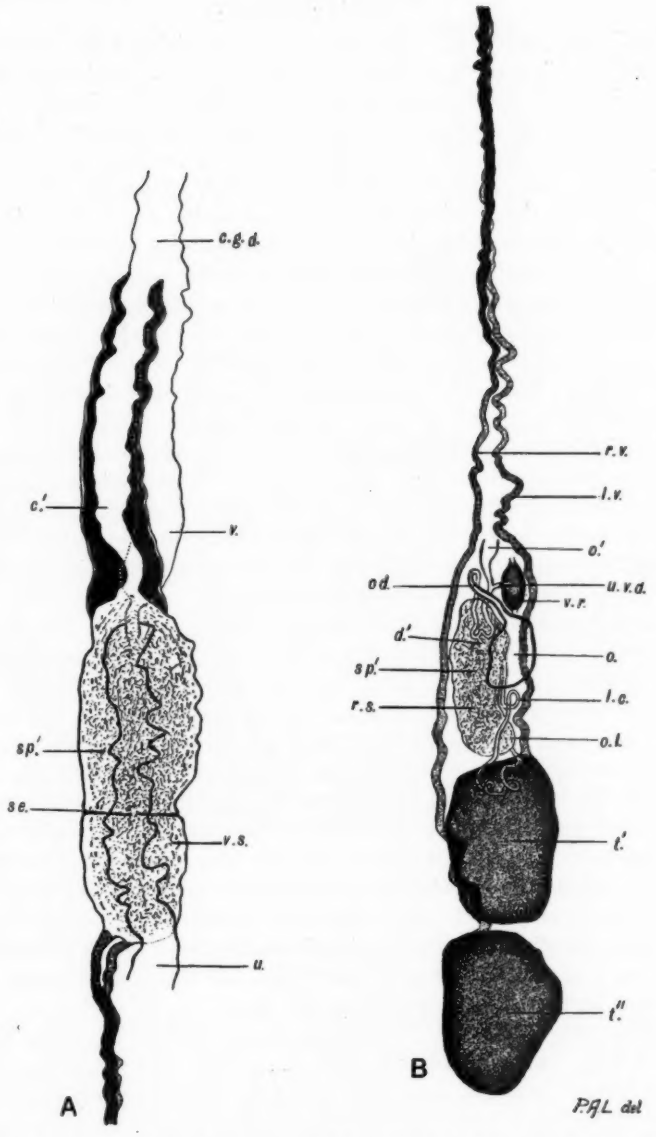
figs. 1, 2 and 4 (*o.s.*)) are 42 to 44 spines (*o.h.'*, *o.h.''*), in the former number (Pl. XXXIX, figs. 1 and 2) two anterior spines, one from both left and right lateral margins, appear to have been accidentally dislodged, an injury which occurs only too frequently among members of the closely related genus *Stephanochasmus* Lss. The 42 or 44 spines are arranged in two interrupted rows of 21 or 23 anteriorly (*o.h.'*) and 21 posteriorly (*o.h.''*), they are flat, almond-shaped, the anterior spines being approximately half the length of the posterior spines. In a specimen 6·2 mm. long (Pl. XXXIX, fig. 2) the mid-dorsal anterior spines measure 0·040 mm. long and 0·022 mm. broad, the lateral spines 0·030–0·034 by 0·015–0·017 mm., the ventral 0·047–0·057 by 0·025–0·026 mm., the spine situated alongside the ventral space being 0·040 by 0·021 mm., the length of the spines of the anterior row is, therefore, approximately twice the breadth. The spines of the posterior row alternate with those of the anterior row; spines placed mid-dorsally measuring 0·083 by 0·032–0·036 mm., laterally 0·051–0·083 by 0·030–0·032 mm., ventrally 0·053–0·057 by 0·026 mm., the innermost ventral spine being 0·043 by 0·025 mm.; the length of the posterior spines being, therefore, little more or less than twice the breadth. The anterior and posterior row of oral spines are interrupted by a ventral space (Pl. XXXIX, fig. 2 (*sp.*)), measuring 0·31 and 0·38 mm. respectively.

The **oral sucker** (Pl. XXXIX, figs. 2 and 4 (*o.s.*)) is transversely oval to circular in outline; in specimens 6·2 mm. (Pl. XXXIX, fig. 2) and 3·8 mm. long (Pl. XXXIX, fig. 4) measuring respectively 0·40 mm. dorso-ventrally by 0·50 mm. broad; and 0·35 by 0·35 mm. The deep basin-like anterior sucker is directed antero-ventrally; it consists of a concentration of numerous muscle fibres embedded in parenchyma and arranged circular-wise, forming its inner and outer boundary, with numerous strong radial connecting muscles.

Numerous backwardly curved claw-like spines cover the body surface (Pl. XXXIX, fig. 2 (*s.*)); first appearing a short distance behind the oral sucker as small fine spines, they become stronger and more numerous approaching the middle length, thence becoming fewer, finally disappearing near the posterior testis to the end of the body. In transverse sections they are seen to be accommodated in epidermal lacuna (Text-figs. 5 and 6 (*e.*)) extending down to the circular muscle fibres.

The **ventral sucker** or acetabulum (Pl. XXXIX, fig. 1 (*a.*)) is situated in the first 1/5th or 1/6th body length; it is circular in outline, measuring in specimens 6·2 mm. and 3·8 mm. long, 0·28 by 0·29 mm. and 0·28 by 0·28 mm. or from 3/5ths to 3/4ths the size of the oral sucker respectively. Histologically (Text-fig. 3 (*a.*)) it resembles the oral sucker.

Cercarian eyes do not appear to be present in this species. The external common genital opening (Pl. XXXIX, fig. 1 (*c.g.o.*)) is situated on the ventral surface immediately in front of the anterior border of the acetabulum (*a.*).



Text-fig. 1. *Dihemistephanus sturionis* n.sp. Schematic view of the reproductive system. A is continuous with B. Ventral aspect. (For lettering see p. 413.)

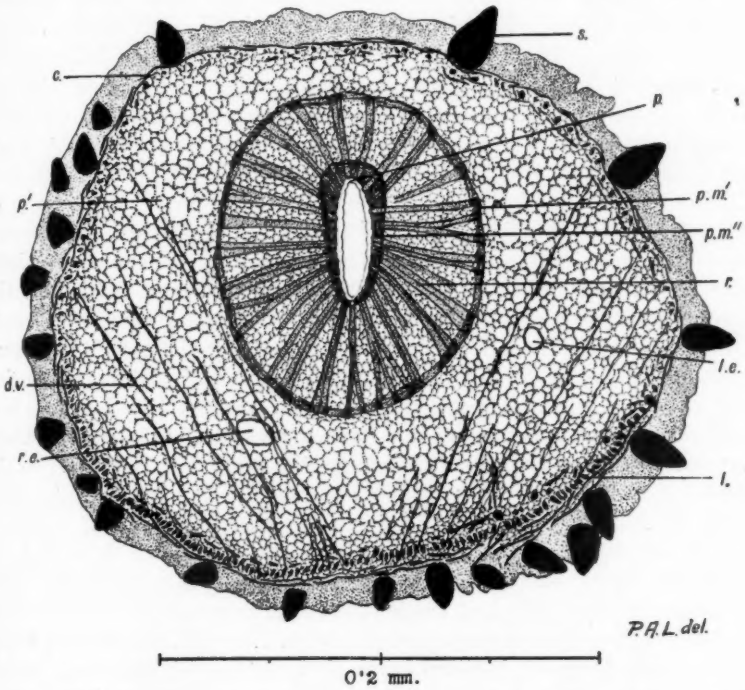
INTERNAL ANATOMY.

Digestive system (Pl. XXXIX, figs. 1, 2, 4 and Text-figs. 2–9). Leading from the mouth (*m.*) is a short prepharynx (*pp.*), 0·072 mm. long in a specimen of 4·5 mm., followed by a strong muscular pharynx (*p.*) oval to circular in outline (Text-fig. 2 (*p.*)), measuring 0·168 by 0·11 by 0·15 mm.; possessing circular (*p.m.'*, *p.m.''*) longitudinal and radial (*r.*) muscles, the inner zone of circular muscle fibres (*p.m.''*) being particularly strongly developed. The detailed structure of the tissue lining the pharyngeal cavity was indiscernible even with 1/12th oil immersion objective, but a non-cellular substance resembling cuticle filled the shallow depressions of its slightly uneven surface, also the extreme dorsal and ventral grooved portion. There follows a short oesophagus (Pl. XXXIX, figs. 1 and 2 (*oe.*)), 0·096 mm. in a worm 4·5 mm. long, dividing at a point approximately midway between the posterior border of the oral sucker and the anterior border of the acetabulum, into a left and right diverticulum. Each diverticulum (Pl. XXXIX, fig. 1 (*d.*) and Text-figs. 3–9 (*l.d.*, *r.d.*)) passes to the posterior extremity of the body; its lumen of variable dimensions ranges from 0·10–0·18 mm. dorso-ventrally, and 0·04–0·12 mm. laterally, appearing largest in the region of the anterior testis; organs of other systems pressing against its lateral sides account for the greater variation in breadth. Amoeboid cells line its lumen, the nuclei being situated basally, the distal projecting pseudopodia appearing enormously vacuolated.

Excretory system. The main excretory canals follow a Y-shaped course. The two long lateral ducts (Text-figs. 2–9 (*l.e.*, *r.e.*), at first narrow, originate in a mid-dorso-ventral position, one on either side of the pharynx; the lumen is lined throughout with thin cuticle. Passing posteriorly one on either side of the oesophagus, they proceed ventralwards in a very convoluted manner along the outer, thence the inner border of each digestive diverticulum; from a point in line with the base of the cirrus to the end of the body they remain for the most part immediately below each diverticulum, eventually entering the ciliated short basal stem portion 0·20 mm. long, filling the space between the two intestinal diverticula and surrounded by accompanying longitudinal muscle fibres; finally terminating by an external pore at the posterior extremity of the body. The diameter of the longitudinal ducts varies from 0·017–0·038 mm. anteriorly, and 0·007–0·010 mm. posteriorly, the oval-shaped lumen of the basal stem portion measuring 0·06 by 0·14 mm.

Reproductive system (Pl. XXXIX, fig. 1, and Text-fig. 1). **Male.** The two longitudinally oval to rhomboidal testes (*t.'*, *t.''*) are situated in the posterior 1/4th to 1/3rd body length, placed one behind the other, medianly, separated by a narrow space which is entirely obliterated in a contracted worm, the opposing borders of the two testes then overlapping. In a worm of 4·5 mm. the posterior border of the second testis is 0·56 mm. or approximately 1/8th the body length from its hind extremity. Both testes are of about equal size, the anterior testis measuring 0·272 by 0·25 by 0·37 mm., the posterior 0·256 by 0·27 by

0·39 mm. in a worm of the above dimension. The vasa deferentia (Text-fig. 1 (*r.v.*, *l.v.*)) are two long ducts of fairly uniform diameter, the right occupying approximately 3/8ths, the left 1/3rd the body length. They arise from the anterior median end of the respective posterior and anterior testis; the right vas deferens (*r.v.*) has a diameter of 0·025 mm. upon leaving the posterior testis (*t.''*), ascending it passes forward above the dorsal surface of the anterior testis (*t.'*), along its right side and immediately below the right longitudinal vitelline duct; thence past the right side of the receptaculum seminis (*r.s.*),

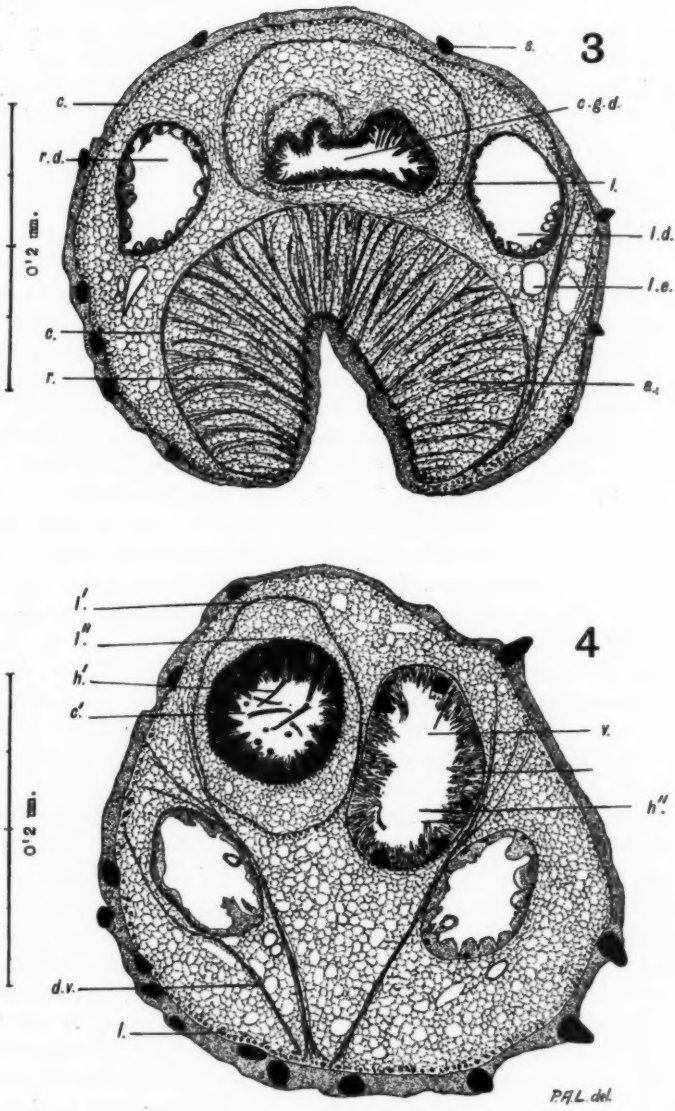


Text-fig. 2. *Dihemistephanus sturionis* n.sp. Transverse section of body in the pharyngeal region. Anterior view.

remaining dorsal and close to the inside of the right digestive diverticulum. The left vas deferens (*l.v.*), having a diameter of 0·019 mm. at its union with the anterior testis (*t.'*), travels forward along the left side of the receptaculum seminis and to the inside of the left digestive diverticulum; thence forward above the ovary (*o.*); continuing its course it passes below the vitelline reservoir (*v.r.*) descending to the ventral side of the left digestive diverticulum; thence gradually ascending it meets the approaching right vas deferens in a dorsal median position. The two vasa deferentia now remain close together, either side by side, or one above the other, their opposing sides often touching, until

they abruptly descend to a mid-dorso-ventral plane to join the vesicula seminalis (*v.s.*). The vesicula seminalis (Pl. XXXIX, fig. 1, also Text-figs. 1 and 5 (*v.s.*)) is a large oval-shaped sac measuring 0·536 by 0·26 by 0·30 mm. in a specimen 4·5 mm. long. It is divided into two unequal-sized chambers by a thin membranous partition (Pl. XXXIX, fig. 1, also Text-fig. 1, (*se.*)), which is perforated by a central circular orifice 0·04 mm. in diameter; around the periphery of the partition are large cells having prominent nuclei and pronouncedly granulated cytoplasm; the anterior chamber is approximately twice the length of the posterior chamber, both being packed with sperm (*sp.'*) and closely invested by numerous broad bands of longitudinal muscle fibres (Text-fig. 5 (*l.*)). Anteriorly the vesicula seminalis gives rise to the base of the cirrus (Pl. XXXIX, fig. 1, also Text-fig. 1 (*c.'*)). From the point of origin to its union with the vagina (*v.*) the cirrus measures 0·504 mm. long in a worm of 4·5 mm. The manner of its fusion with the vagina to form the common genital duct (Text-fig. 3 (*c.g.d.*)) suggests that it is incapable of protrusion. Its lumen (Text-fig. 4 (*c.'*)) is circular in outline, varying in diameter from 0·045 mm. basally to 0·100 mm. distally; at first its cavity is almost completely blocked by squamose cells loosely held together which are replaced later by numerous anteriorly directed strong spines (*h.'*), some measuring 0·053 mm. long, and having the appearance set forth in Pl. XXXIX, fig. 3. An outer and inner zone of longitudinal muscles (Text-fig. 4 (*l.', l.''*)) accompany the cirrus throughout its course.

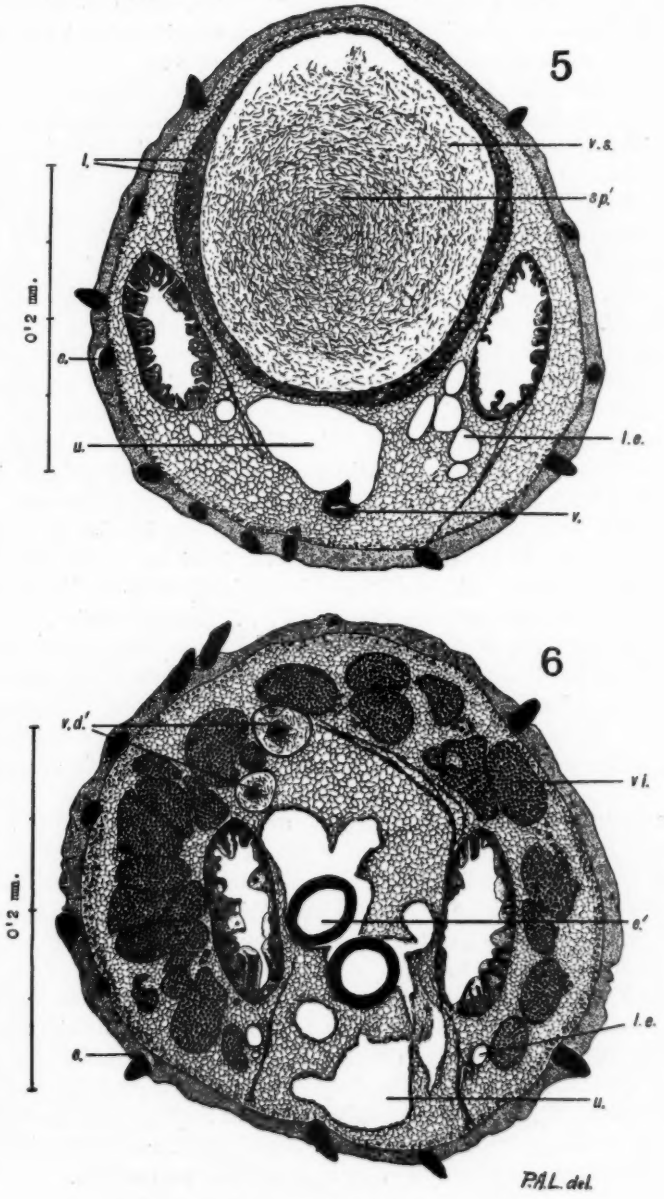
Female. The ovary (Text-fig. 1 (*o.*)) is spherical to oval in outline, measuring 0·120 by 0·11 by 0·18 mm. in a specimen 4·5 mm. long, or a trifle less than half the size of the testes. It is situated in front of, and close to the anterior testis (*t'.*), towards the ventral side of the worm (Text-fig. 8 (*o.*)), remaining to the inside of the left digestive diverticulum and the left side of the receptaculum seminis (*r.s.*). The oviduct (*od.*) is circular in transverse section, having a diameter of 0·017 mm. at its union with the anterior end of the ovary. It travels a short distance forward, then, turning abruptly upon itself (Text-figs. 1, 7 (*od.*)), proceeds backward, passing above the receptaculum seminis (*r.s.*), and receiving therefrom a short duct (Text-figs. 1, 8 (*d.'*)), thence completely turns about to resume its forward course for a distance of 0·112 mm. Before the oviduct joins the ootype (*o.'*) it receives the unpaired vitelline duct (Text-figs. 1, 7 (*u.v.d.*)) from the vitelline reservoir (*v.r.*) situated to its left. The ootype (*o.'*) is 0·05 mm. long with the maximum diameter of 0·030 mm. It is lined with a glandular-like tissue (Text-fig. 7 (*o.'*)) whose retarded development at four distinct points imparts a tetraradiate outline to the lumen. Numerous large and readily stainable cells (*s.g.*), possessing prominent nuclei, are irregularly disposed around the ootype; these probably represent the shell gland. The ootype passes on to the capacious uterus. The voluminous uterus (Pl. XXXIX, fig. 1, also Text-figs. 5–9 (*u.*)) is packed with eggs throughout, and extends in a convoluted manner anteriorly to a point parallel with the hind end of the vesicula seminalis (*v.s.*) and posteriorly to the hind extremity of the body, occupying a considerable volume of the body as seen in the



Text-figs. 3, 4. *Dihemistephanus sturionis* n.sp.

Text-fig. 3. Transverse section of body in the acetabular region. Anterior view.
Text-fig. 4. Transverse section in the region of the vagina and cirrus. Anterior view.

accompanying figures of transverse sections. The uterus (Text-fig. 1 (*u.*)) passes under the vesicula seminalis (*v.s.*) parallel with its median longitudinal axis as a fairly straight duct, its lumen measuring 0·094 by 0·057 mm., abruptly diminishing to a diameter of 0·02 mm. to indicate the beginning of the vagina. The vagina (Text-fig. 1, also Pl. XXXIX, fig. 1 (*v.*)) is 0·560 mm. long, originating at a point a little posterior to the base of the cirrus (*c.'*) and terminating in a line parallel with the posterior region of the acetabulum (*a.*), where it fuses with the cirrus forming the common genital duct (*c.g.d.*). It runs parallel with the cirrus (*c.'*) along its left side (Text-fig. 4 (*v.*)). Its lumen, at first circular in outline and small, becomes larger and of a somewhat oval shape, measuring 0·09 by 0·12 mm. It is lined with numerous anteriorly directed pointed spines (*h.''*), interspersed with cilia (*c.''*); the spines have a maximum length of 0·05 mm.; some possess graceful urn-shaped theca (Text-fig. 10). The totally dissimilar affinity for stains displayed by the spine and theca, respectively, suggest a difference in chemical composition, the former probably consisting of chitin, the latter gelatin or mucilage. It is noteworthy that, while a great similarity exists between the cirrus spines and the vaginal spines, it is only certain of the latter which possess the theca, thereby suggesting that they serve for the easier emission of eggs. The atrium or common genital duct (Text-fig. 1, also Pl. XXXIX, fig. 1 (*c.g.d.*)) is 0·192 mm. long in a worm of 4·5 mm. Its lumen (Text-fig. 3 (*c.g.d.*)) is transversely oval in section, measuring 0·11 by 0·026 mm., situated immediately above the median longitudinal axis of the acetabulum (*a.*); it abruptly descends to the ventral surface immediately in front of the anterior border of the acetabulum, terminating at the slightly crescentric external common genital opening (Pl. XXXIX, fig. 1 (*c.g.o.*)), measuring 0·019 by 0·083 mm. In specimens mounted *in toto* and dorso-ventrally compressed, the solid nature of the muscular acetabulum displaces the common genital duct to either side of its lateral margins, as seen in the worm figured. Numerous well-developed longitudinal muscle fibres (Text-fig. 3 (*l.*)) disposed around the periphery of the atrium accompany it throughout its length. The receptaculum seminis (Text-fig. 1 (*r.s.*)) is 0·256 mm. long in a worm of 4·5 mm.; it originated in a line parallel with the front border of the anterior testis (*t.'*) and passes forward along the median longitudinal axis of the body, terminating at a point approximately in line with the hind border of the vitelline reservoir (*v.r.*). Its lumen (Text-figs. 8, 9 (*r.s.*)) is oval to circular in outline, measuring 0·13 by 0·23 mm., and situated to the right side of the ovary (*o.*). It is packed with sperm (*sp.'*) and is connected to a Laurer's canal (*l.c.*). The external opening of the Laurer's canal (Text-figs. 1 and 9 (*o.l.*)) is situated on the dorsal median surface of the body, almost in line with the posterior border of the receptaculum seminis (*r.s.*). The external orifice leads to a duct 0·019 mm. in diameter and lined with thick cuticle; the convoluted duct (Text-fig. 1 (*l.c.*)) travels a short distance backward, passing above the dorsal margin of the anterior testis (*t.'*); thence losing its cuticular lining it turns completely about, proceeding forward to



Text-figs. 5, 6. *Dihemistephanus sturionis* n.sp.

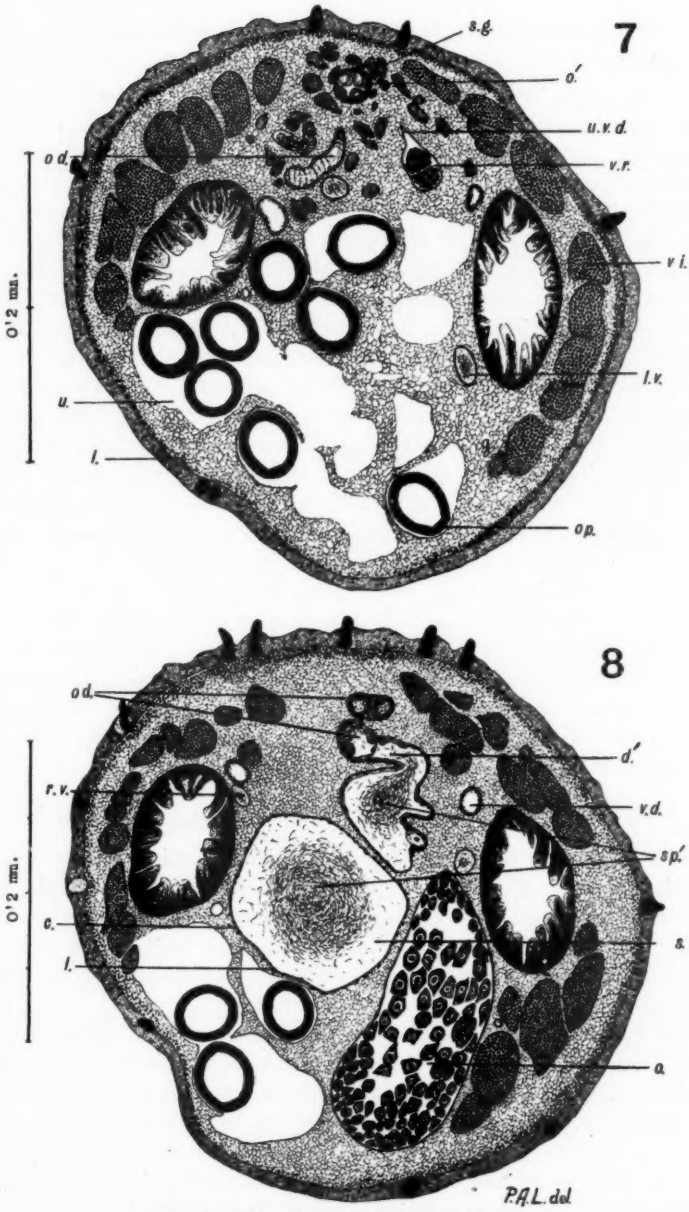
Text-fig. 5. Transverse section in the region of the vesicula seminalis. Anterior view.
Text-fig. 6. Transverse section in region of vasa deferentia. Anterior view.

join the receptaculum seminis (*r.s.*) along its left side, within its anterior half. Bands of circular and longitudinal muscle fibres (Text-fig. 8 (*c.*) (*l.*)) closely invest the receptaculum seminis throughout its course.

The vitelline system (Pl. XXXIX, fig. 1 (*vi.*)) consists of numerous follicles scattered freely throughout the posterior 5/8ths body length, first appearing on the right side of the vesicula seminalis (*v.s.*) in a line with its middle length, the follicles of the left side appearing near its posterior border. The follicles of either side increase in number and gradually extend dorsalward, eventually meeting at a point approximately 0·16 mm. behind the posterior border of the vesicula seminalis (Text-fig. 6 (*vi.*)), thence occupying the peripheral regions of the body, forming a "horse-shoe" pattern, the open end of which faces ventralward. The dorsal follicles disappear in the vicinity of the ootype (*o.'*) (Text-figs. 7, 8 and 9 (*vi.*)), re-appearing in the narrow space between the two testes, thence disappear; finally re-appearing in a line with the hind region of the posterior testis, alone persisting in the hindermost part of the body, finally vanishing at a point approximately midway along the length of the basal excretory duct. The lateral follicles persist in the vicinity of both testes, finally disappearing behind the posterior testis. The vitelline reservoir (Text-fig. 1 (*v.r.*)) is 0·080 mm. long, originating in a line parallel with the posterior end of the ootype (*o.'*) and terminating at the front end of the ovary (*o.*); it lies to the left of the oviduct (*od.*) and immediately to the inside of the left digestive diverticulum. Its lumen (Text-fig. 7 (*v.r.*)) is oval in outline, measuring 0·045 by 0·075 mm.; it receives yolk material conveyed by main anterior and posterior longitudinal ducts situated along the right and left sides of the body. An unpaired vitelline duct (Text-figs. 1 and 7 (*u.v.d.*)) arises from the dorsal side of the vitelline reservoir along its inner side at a point approximately 2/5ths behind its front end; it passes dorsalward to join the oviduct (*od.*).

The mature eggs (Text-figs. 6–9 (*e.'*)) are oval, circular in cross-section, measuring from 0·043–0·049 by 0·034–0·043 mm., and possessing rich yellow shells, 0·007–0·008 mm. thick. When viewed laterally, two inwardly converging lines can be seen at that end where the shell is thinnest, evidently an operculum (*op.*); eggs are numerous, distending the voluminous uterus, while a few are scattered throughout the vagina.

Muscular system. This is well developed in *Dihemistephanus sturionis* n.sp. Muscles associated with other systems have already been dealt with when describing the various systems above. The body muscles consist of an outer layer of circular muscle fibres (Text-fig. 2 (*c.*)) and an inner layer of longitudinal muscles (*l.*), the latter increasing in bulk as they approach the posterior extremity of the body; the dorso-ventral muscles (*d.v.*), on either side of the median line, converge slightly as they approach the ventral side.



Text-figs. 7, 8. *Dihemistephanus sturionis* n.sp.

Text-fig. 7. Transverse section in the region of the ootype. Anterior view.
Text-fig. 8. Transverse section in the region of the ovary. Anterior view.

Systematic position of the genus **Dihemistephanus** Lss.

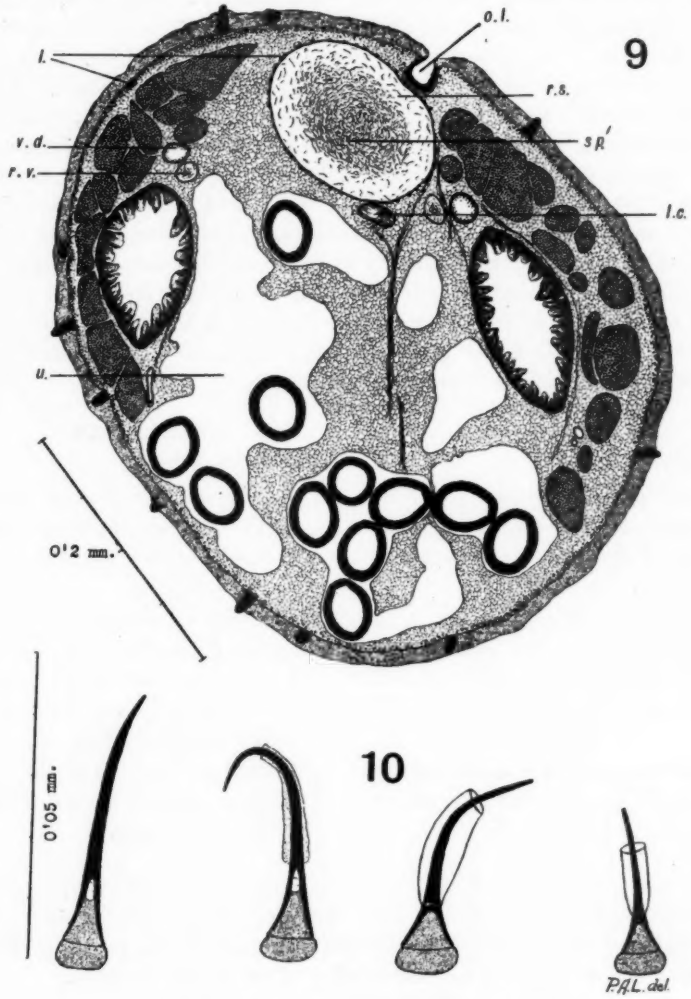
Stossich in 1896 described a trematode under the name of *Echinostomum lydiae* Stoss. from the *Orthagoriscus mola* (Sun-fish). Stossich's description led Looss to believe that the parasite really belonged to the genus *Stephanochasmus* Lss. Later, however, Looss 1901 had an opportunity of examining original material lent by Stossich, whereupon he concluded he was mistaken in assigning the trematode to the genus *Stephanochasmus* Lss., and was of opinion that it represented a new genus which he named *Dihemistephanus*. Looss complained that the material at his disposal was not very abundant, and the condition of preservation of most specimens left much to be desired; the oral and body spines being preserved in only a single specimen. The oral spines, according to Looss, provide one of the outstanding diagnostic features of the genus, distinguishing it from the closely related genus *Stephanochasmus*, viz. the oral spines in *Stephanochasmus* are arranged in two uninterrupted rows, whereas in *Dihemistephanus* the rows are interrupted by a wide ventral space. Nevertheless, it is worthy of note that Looss discovered a small ventral interruption of the oral spines in both *Stephanochasmus cesticillus* (Molin) and *S. bicoronatus* (Stossich), in which there exists a slightly wider interval between the most ventral spines than between the remaining front row spines, and, whereas the spines of the back row strictly alternate with those of the front row, the spine which should normally appear opposite the rather wider ventral interval is either very diminutive or entirely absent. For this reason one may safely postulate a close relationship between the genera *Stephanochasmus* and *Dihemistephanus* which share a number of common features. Both genera belong to the Sub-family *Echinostominae* Lss., Family *Fasciolidae* Rail., Sub-order *Malacocotylea* Mont., Order *Trematoda* Rud.

Diagnostic features of the genus **Dihemistephanus** Lss.

Owing to the bad preservation of the material examined, Looss 1901 was compelled to rely mainly upon external characters in diagnosing the genus *Dihemistephanus*. The four living specimens of *D. sturionis* Little, examined by the writer, provided, on the contrary, excellent material for fixing and sectioning, their anatomical features serving to complete the general account of the genus.

Body frail, capable of considerable elongation during life, especially the pre-acetabular region; for this reason a consideration of measurements alone is misleading. Length 3·5–6·2 mm. in fixed and partially contracted specimens; width 0·49–0·69 mm. (0·8 mm. Looss) or 1/5th to 1/9th the length; oval to circular in cross-section. **Suckers** either equal or the acetabulum 2/5ths to 3/4ths the size of oral sucker; acetabulum occurs within the anterior 1/5th–1/6th length (in partially contracted specimens): according to Looss 1901 and Stossich 1896 it is placed as far back as the beginning of the posterior half. **Oral sucker** partially surrounded by numerous large spines arranged in two

rows, interrupted by a wide ventral space. **Skin** strongly armed with spines disappearing posteriorly. **Prepharynx** and strong **pharynx** present; short **oesophagus** divides in front of the acetabulum, forming two intestinal diverti-



Text-figs. 9, 10. *Dihemistephanus sturionis* n.sp.

Text-fig. 9. Transverse section in the region of the receptaculum seminis. Anterior view.
Text-fig. 10. Vaginal hooks.

cula extending almost to the posterior extremity. **Excretory system** Y-shaped, short stem and two long convoluted arms extending to the level of the pharynx. **Common genital aperture** situated immediately in front of the acetabulum,

leading into a **genital atrium** which stretches to the posterior region of the acetabulum. **Testes** two, oval to rhomboidal, median, placed within the posterior half of the body, one behind the other, their edges touching or separated by a narrow interval; **vasa deferentia** two long ducts, entering a **vesicula seminalis** situated within the anterior half of the body and divided into two chambers by a septum possessing a central orifice. **Cirrus** long, non-protrusible, armed with internal strong, pointed spines. **Ovary** spherical, median, anterior to testes; convoluted oviduct, small ootype, voluminous uterus, **vagina** about same length as cirrus with which it fuses to form a common genital duct; numerous vaginal spines present. **Laurer's canal** and **receptaculum seminis** present. **Diffuse shell gland** occurs in the region of ootype and oviduct. **Vitelline follicles** abundant, extending from the vesicula seminalis to the posterior extremity of body; chiefly confined to lateral and dorsal regions; vitelline reservoir placed slightly anterior and dorsal to ovary. **Eggs** numerous, thickish yellow shells, operculated. The two representatives of the genus are *D. lydiae* (Stossich) and *D. sturionis* Little, possessing the following distinguishing features:

D. lydiae (Stossich)	*D. sturionis* Little
Length 4 mm.	*Length* 3·5–6·2 mm.
Greatest breadth 0·8 mm. or 1/5th length, at the height of acetabulum, gradually decreasing posteriorly.	*Greatest breadth* 0·49–0·69 mm. or 1/9th–1/7th length, some distance behind the acetabulum.
Oral spines 39, cylindrical, long, spike-like: anterior row of 20, posterior row of 19, spines of both rows about the same size.	*Oral spines* 44, flat, almond-shaped, length about twice the breadth; anterior row of 21–23, posterior row of 21, which are twice the size of the anterior spines.
Acetabulum same size as oral sucker, situated at front end of posterior half of body.	*Acetabulum* 3/5ths–3/4ths size of oral sucker, situated in first 1/5th or 1/6th body length.

In conclusion I wish to express my cordial thanks to Major T. R. Ronald of the Consolidated Fisheries, Ltd., Swansea, for permission to examine material, and also to Mr H. Leadbetter, salesman to the company, for his courtesy and help during the examination of the Royal Sturgeon.

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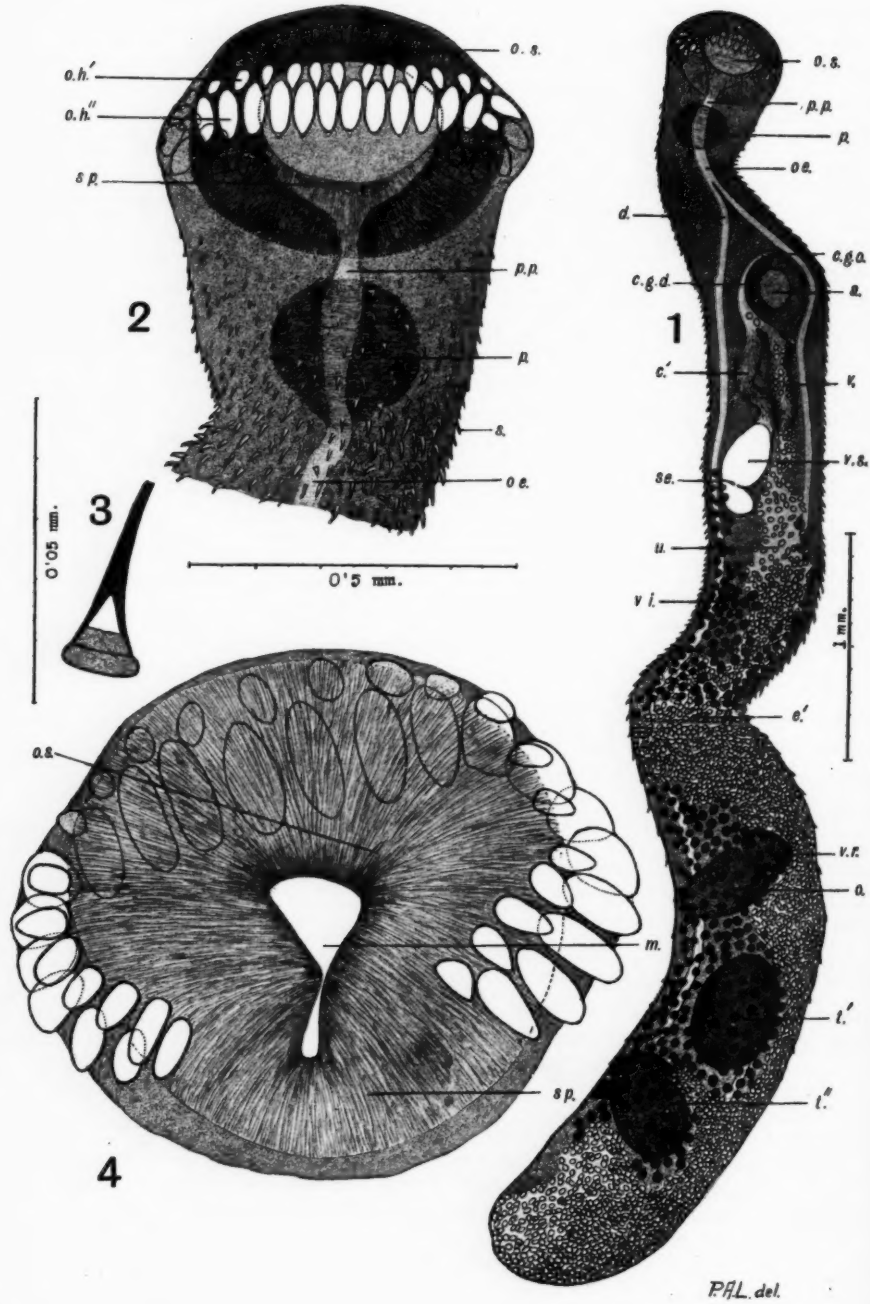
Stossich, M. (1896). Elminti trovati in un Orthagoriscus mola. *Boll. Soc. Adriatica.* **17**, 4, Fig. 1.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2
3
0·05 mm.
0·5 mm.
o. s.
p.p.
p.
oe.
d.
c.g.a.
c.g.d.
a.
1
c.'
v.
v.s.
se.
u.
v.l.
1 mm.
e.'
o.s.
v.r.
a.
m.
l.'
l.''
s.p.
4
P.A.L. del.

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EXPLANATION OF PLATE XXXIX.

Dihemistephanus sturionis n.sp.

Fig. 1. Semi-contracted worm 6·2 mm. long, mounted *in toto*. Ventral view.
Fig. 2. Dorsal view of the anterior end of specimen represented in Fig. 1.
Fig. 3. A hook located in the lumen of the cirrus. Distal end incomplete.
Fig. 4. Antero-ventral view of the oral sucker of a worm 3·8 mm. long.

KEY TO LETTERING OF PLATE XXXIX AND TEXT-FIGURES.

a. Acetabulum.
c. Circular muscles.
c.' Cirrus.
c.'' Cilia.
c.g.d. Common genital duct.
c.g.o. Common genital opening.
d. Digestive diverticulum.
d.' Duct connecting receptaculum seminis to oviduct.
d.v. Dorso-ventral muscles.
e. Epidermal lacuna.
e.' Egg.
h.' Broken hooks in cirrus.
h.'' Broken vaginal hooks.
l. Longitudinal muscles.
l.' Outer zone of longitudinal muscles of cirrus.
l.'' Inner zone of longitudinal muscles of cirrus.
l.c. Laurer's canal.
l.d. Left diverticulum.
l.e. Left longitudinal excretory duct.
l.v. Left vas deferens.
m. Mouth.
o. Ovary.
o.' Ootype.
od. Oviduct.
oe. Oesophagus.
o.h.' Anterior row of oral hooks.
o.h.'' Posterior row of oral hooks.
o.l. External opening of Laurer's canal.
op. Operculum of egg.
o.s. Oral sucker.
p. Pharynx.
p.' Parenchyma.
p.m.' Outer zone of pharyngeal circular muscles.
p.m.'' Inner zone of pharyngeal circular muscles.
pp. Prepharynx.
r. Radial muscles.
r.d. Right diverticulum.
r.e. Right longitudinal excretory duct.
r.s. Receptaculum seminis.
r.v. Right vas deferens.
s. Body spine.
se. Septum.
s.g. Shell gland.
sp. Ventral space.
sp.' Sperm.
t.' Anterior testis.
t.'' Posterior testis.
u. Uterus.
u.v.d. Unpaired vitelline duct.
v. Vagina.
v.d. Longitudinal vitelline duct.
v.d.' Vasa deferentia.
vi. Vitellaria.
v.r. Vitelline reservoir.
v.s. Vesicula seminalis.

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"RED-CELL ADHESION" IN TRYPANOSOMIASIS OF MAN AND ANIMALS.

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I. INTRODUCTION.

It was Major H. C. Brown of the Wellcome Bureau of Scientific Research who first drew the attention of one of us to the possible value of the Rieckenberg reaction in the study of trypanosomiasis. Pressure of other work precluded any investigation until February 1929, when a visit to Uganda of Dr W. B. Johnson, then Director of the Sherifuri Institute, Nigeria, led to a revival of our interest in this phenomenon, and shortly afterwards Johnson and Lester's paper reached us (1928). A perusal of their experiments left us no alternative. For a piece of work of this kind the facilities at the Entebbe Institute are unique.

Strains of both *T. gambiense* and *T. rhodesiense*, all recovered originally from hosts infected by wild tsetse, have been maintained under observation, some for several years. Passage from mammal to mammal has been effected chiefly, where not exclusively, by cyclically infected *G. palpalis*. The available experimental animals comprise a considerable variety of species, including monkeys, guinea-pigs, sheep, goats, calves and a wild pig, the history of every infection being known in detail: some of these had been infected with *T. gambiense* for over two years. In addition, human cases of trypanosomiasis are obtainable from various infected areas in the Protectorate, and a small well-equipped hospital attached to the Laboratory enables us to carry out an intensive study of a few selected cases on the spot.

It was decided therefore, in spite of a serious shortage of staff[1], to fit into our programme a study of the adhesion test as applied mainly to infections due to *T. gambiense* and *T. rhodesiense*, examining as much as possible of the certified material at our command.

Our experience, as set forth below, differs in several respects from that of previous workers. The great majority of the trypanosome strains investigated at Entebbe were never subjected to drugs. Our "relapse strains" owe their development to the action exerted upon them by the unaided tissues of the host. This peculiarity attaches to the great majority of the *T. gambiense* "immune" bloods investigated. In the case of *T. rhodesiense*, whose virulence is considerably greater, it was necessary to resort to Bayer 205, partly to get rid of the animal's own trypanosomes and partly to ensure the development of "immune bodies." Here, however, we employed single sterilising doses, and in no case did an animal so treated relapse spontaneously.

We fully realise that a great deal of work remains to be done upon a very uncommon material. The experiments of this paper represent the preliminary clearing of the ground and the recognition of certain cardinal limitations to the utility of the test in diagnosis. We have reached the conviction that the phenomena we have observed are manifestations of definite biological processes, and are not due to accident or purely to physical causes; they appear to us to be fundamentally of the same nature as those already described as "adhesion phenomena" by a number of other workers in different countries.

In this paper we do not attempt to make a complete study of the mechanism of the adhesion phenomenon, but wish merely to define its limitations as a clinical test and to compare our results, in so far as they are comparable, with those of previous workers.

A perusal of Johnson and Lester's paper leaves the impression that the test is the "Open sesame" to all problems of diagnosis of chronic trypanosomiasis. Our experience hitherto has not confirmed this opinion, and we have

[1] The staff of the Institute was, at the time, reduced to the two of us, one of whom was also responsible for the direction of the Laboratory Services Division of the Medical Department of the Protectorate. Furthermore, our existing programme, based on the indications put forward by the League of Nations Expert Committee, demanded constant personal attention, and the loss in June of our Laboratory Assistant was a serious handicap.

considered it expedient to support our case with a reasonable weight of detailed evidence.

It is probable that more work on the adhesion phenomenon has been done and published than we are aware of. The literature available at Entebbe is very limited and we acknowledge with gratitude assistance in this direction from the Health Section of the League of Nations, from Dr H. H. Scott of the Committee of Civil Research and from Miss Muriel Robertson of the Lister Institute. The list of references shows all the papers we have seen. The bulk of the experimental work contained in these papers on von Rieckenberg's phenomenon in trypanosomiasis has been done with highly virulent laboratory strains of pathogenic trypanosomes such as *T. brucei* and *T. equiperdum*, the like of which have never been seen in Uganda. With these fierce strains the results were, in the main, similar to those obtained by Brown and Davis and others with spirochaetes. Definite serological differences were noted between "passage" and "relapse" strains, and also between different "relapse" variants from a single "passage" strain.

The delicacy of discrimination revealed by the experiments of the German workers, especially those of Ritz, seemed to us at first glance to augur badly for the success of the adhesion test in the diagnosis of the chronic trypanosomiases with which we have to do in Uganda. The trypanosomes at our disposal included several different strains of *T. gambiense*, one of *T. rhodesiense*, and one of the Damba trypanosome whose recognised attributes seem to place it midway between a typical *T. gambiense* and a typical *T. brucei* (Lavier, 1928). None of these strains resemble in virulence those in the European laboratories. All were originally isolated from men or animals that were in their turn "naturally" infected in a fly belt. The *T. gambiense* strains, even after three years of maintenance, still possess a comparatively low degree of virulence. We are, in fact, doing all that we can to increase this virulence, so far without much success. The Damba trypanosome, though more virulent, takes six or eight weeks to kill a guinea-pig or monkey, and during this time the number of parasites in the peripheral blood varies from day to day and week to week, being often so small in guinea-pigs that none can be found by the ordinary method of stained thick-film examination. In *T. rhodesiense* we have an organism that is definitely more pathogenic, and yet even with this trypanosome the average duration of life in fourteen infected guinea-pigs and nine monkeys, all untreated, was forty-seven and thirty-two days respectively.

If then the test be in truth as delicate as the experiments of the German workers seem to show (Ritz obtained twenty-two serologically different relapse strains from the same mouse), there appears little hope of its successful application in the diagnosis of trypanosomiasis in naturally infected communities of men and animals. Our early attempts at demonstrating the phenomenon of adhesion tended to confirm these doubts. Using centrifuged citrated "immune" and trypanosome plasmas under high-power magnification and dark-ground illumination, our results with both *T. gambiense* and *T. rhode-*

siense were entirely inconclusive. Hardly any adhesion was observed either of blood dust or of platelets, and that little was equalled in the control preparations. Occasionally single red cells were seen adherent both in the test and its controls. The arrival of Johnson and Lester's paper opened up new vistas. We followed their technique but still with unconvincing results. Finally, lest some technical error were responsible for our repeated failures, a letter was despatched to Brown and Davis describing our procedure and asking for advice. Satisfied at last that the technique was not to blame, the inevitable conclusion emerged that the serological homology between our reagents—the "immune" and the "trypanosome" plasmas—was not sufficiently close to give a positive test. We then tried the method recommended by Leupold (1928), and at last we obtained our first success. On adding to a drop of citrate one drop of blood from a monkey infected with *T. rhodesiense*, which had a few days before received a curative dose of Bayer 205, and one drop from another monkey heavily infected with the same strain, every trypanosome was seen to have red blood corpuscles sticking to it. The phenomenon was easily visible under a 1/6th objective with a low eyepiece. All the trypanosomes were completely enveloped in red cells, suggesting immediately a caddis worm and leading to our use of the term "caddis-form" throughout this paper; others had a few corpuscles scattered along their length; others again only one. No other adherent body was seen.

In the literature at our disposal there is only one definite reference (Leupold, 1928) to the adhesion of red blood corpuscles to trypanosomes. Blood platelets, blood dust and inert foreign indicators are all mentioned as suitable for demonstrating the phenomenon. Leupold considers the adhesion phenomenon to be a means of defence on the part of the trypanosome against the effects of the immune serum, and believes that the blood platelets are selected principally because of their convenience and low specific gravity. She goes on to say, however, that in the course of her experiments "in positiven Präparaten häufig auch einzelne rote Blutkörperchen an den Trypanosomen hafteten und von ihnen hin und her gepeitscht wurden, eine Beobachtung, die es wahrscheinlich erscheinen lässt, dass die Ursache der Fixierung der Blutelemente an der Flagellatenzelle vom Trypanosoma selbst ausgeht, das die molekular beweglichen und schwereren Erythrocyten nur vereinzelt zu fesseln vermag." We shall return to a study of the mechanism of red-cell adhesion in Part III of this paper. Suffice it to say here that the choice of blood platelets or of red cells is not merely dependent on specific gravity. In all our preparations manifesting exclusively red-cell adhesion there were plenty of platelets to be seen among the cells, but they remained apparently untouched.

It is difficult to believe that the particular manifestation of adhesiveness that we have just described has not been noted by other workers. As will be seen below, it was of regular occurrence with our strains, very much commoner in fact than platelet or blood dust adhesion, both of which phenomena were of comparatively rare occurrence in our experiments, though appearing

regularly in certain definite circumstances. We suggest an explanation of this in Part III. On the other hand, it seems plain that none of the investigators quoted above can have witnessed the phenomenon of red-cell adhesion to the same extent that we have and yet not have been constrained explicitly to describe it. The appearance of the "caddis forms" is so striking that a positive reaction can be easily recognised under a 2/3rd objective, and the simplicity of the test is astonishing. Be it noted in passing that the great majority of our predecessors employed small laboratory rodents, whereas our principal experimental animal was the *Cercopithecus* monkey. On account of the novelty of our findings it was therefore necessary to determine whether this manifestation was really specific, and if so to what degree; and it is to attain this knowledge that the following examinations have been carried out.

(*a*) *Special terms employed.*

For the sake of brevity, throughout this paper the term "adhesin" is used for the substance responsible for the adhesion phenomenon. In like manner the abbreviation "tryp." is used in indicating the trypanosome-containing blood. A test preparation is, then, the admixture of adhesin blood and tryp. blood.

In certain tests the adhesin blood may have contained rare trypanosomes, but their number was never sufficient to interfere in any way with the reading of the test. Thus most of the animals referred to in Table I have never been treated with drugs, but as all these were infected with *T. gambiense* and were only tested when trypanosomes were rare the point is unimportant.

In addition to true red-cell adhesion there are other phenomena that demand attention if only to avoid confusion in the interpretation of the test. These are non-specific adhesion, pseudo-adhesion, agglomeration of trypanosomes and agglutination of the red cells.

(*b*) *Non-specific adhesion.*

Here we are on difficult ground. There is never any doubt about a strong positive, and there is also no question that the degree of affinity between the trypanosome and the adhesin varies greatly. Similar variations were noted by Leupold in connection with relapse strains. In our experiments the intensity of the response is indicated by the system of symbols given below. It will be seen, too, that the variability is very great; from day to day the results of the test in the case of the same two animals may differ considerably. It is tempting to show these differences in exact percentages as they are noted in the day-book, the more so as instances are not uncommon where the affinity of an adhesin plasma for a certain trypanosome remains remarkably constant over a considerable period. But no useful purpose would be served by a rigid observance of such procedure at this stage in our acquaintance with the test. When percentages are given they merely serve as a secondary indication of the degree of the reaction within the wide limits covered by the symbols "tr" and +, *i.e.* from 10 per cent. to 49 per cent.

The study of controls has revealed the fact that the blood of some individual animals that have never been infected with trypanosomes may possess the power, though in a very restricted degree, of producing typical adhesion of red blood corpuscles to trypanosomes.

In such a preparation, among an overwhelming majority of clear trypanosomes, an occasional "caddis form" will be seen.

Once this point was established it became necessary to try to define the limits of this non-specific reaction, leaving at the same time a wide margin of safety. As the result of a number of such control experiments with animals that had never suffered from trypanosomiasis we have decided to exclude all results below 10 per cent. as of doubtful significance. This is approximately two and a half times greater than the highest value ever obtained from a known negative blood.

As will be seen later, the significance of the reaction of a blood with different "species" of trypanosomes is not easy to understand. Here unfortunately our controls are limited, owing to the impossibility of obtaining a sufficient variety of strains.

(*c*) *Pseudo-adhesion.*

Trypanosomes may become entangled in small agglomerations of cells and after a while break free. Pseudo-adhesion is, however, most noticeable when single corpuscles become involved in the movement of a parasite. Often in such cases the lashing movements of the flagellum titillate for a considerable time one particular cell which for a while appears to move in unison with the parasite. But if watched for a short time it will be seen that the movements of the cell are neither strictly synchronous nor continuous and that adjacent cells take up the tale, and eventually it is seen that the attachment is only apparent.

(*d*) *Agglomeration and agglutination.*

Agglomeration of trypanosomes, common in serological experiments with these organisms and mentioned as a nuisance by Leupold, we have seen from time to time, but cannot define its significance. There remains one important point, namely the occasional occurrence of agglutination of the red cells when certain bloods are mixed. This phenomenon to some extent interferes with the manifestation of adhesion. The tendency of the cells to stick together itself suggests a fallacy, and in addition there is the possibility of the trypanosomes being mechanically entrapped by the gathering cell masses and so simulating adhesion. By lateral movements of the coverslip a mild degree of auto-agglutination may often be broken up, and in parts, at any rate, of the film the cells found to be evenly distributed. One hesitates, however, to proceed with such preparations, especially when human blood is involved. In such circumstances the border line reactions between true and non-specific adhesion are sometimes difficult to define. This agglutination of red cells is doubtless associated with the different types of blood groups, but there has been as yet no opportunity to examine this question thoroughly. The blood of certain

individual monkeys and guinea-pigs possesses this property to a high degree, and more especially calf-blood. But before leaving this point it should be noted that auto-agglutination, *per se*, does not necessarily cause the adhesion of red cells to trypanosomes. The danger is in the case of border-line preparations; strong positives and complete negatives offer no real difficulty.

(*e*) *Technique and description of red-cell adhesion.*

Equal parts of each blood and 2 per cent. citrate saline were mixed in a small tube. With a fine pipette equal quantities of the adhesin blood and the tryp. blood were then mixed on a slide, and from the resulting large drop two coverslip preparations were made upon one slide. We found it convenient to use for this purpose large coverslips. All glassware was carefully cleaned before use. The thickness of the preparation is a matter of some importance. The cells should be evenly distributed, not superimposed on one another nor flattened by the coverslip, but freely movable. The thick massed parts around the edge are to be avoided; here the trypanosomes are liable to injury from desiccation. When the coverslip has been lowered into position the slide must be left to stand for ten minutes before being examined. The adhesin blood can be kept for four or five hours on the bench without impairing its activity, but the tryp. blood should not be kept for longer than one hour. The actual preparation should be examined without undue delay after the ten minutes have elapsed, although once adhesion has taken place lateral movement of the coverslip and replacing it do not detach the corpuscles.

Parts of the film where the cells are superimposed and massed together are to be avoided, likewise very thin areas where the red cells are flattened out and beginning to feel the effect of drying. In the thick parts there is a tendency to pseudo-adhesion and an accurate view is impossible; in the thin parts the trypanosomes do not seem to have a fair chance to pick up the compressed corpuscles.

In a strongly positive preparation the corpuscles begin to adhere immediately, and every trypanosome will rapidly acquire a load of cells. In such a preparation, when the parasites are scanty—for instance one to eight or ten fields with the 1/6th objective and No. 2 eyepiece, or less—the scattered trypanosomes proclaim their whereabouts by the movements of the attached corpuscles which wave about like clusters of tiny balloons. The "caddis forms" are also very striking objects in a properly prepared slide.

More difficult to judge are the cases where single corpuscles are adherent. In cases of genuine as distinct from temporary or pseudo-adhesion the corpuscle, by its jerky or rotatory movements, reflects faithfully every movement of the trypanosome and is never still. A curious feature of single-celled adhesion is the way the trypanosome from time to time slips along the surface of the cell, from the tip of its posterior end to the extreme point of its flagellum, always seeming on the point of breaking away but never actually losing contact.

It will be seen that excepting the small group of experiments where platelet adhesion was the rule, the great majority of the preparations examined showed red-cell adhesion almost exclusively. A common type is a small bunch of two or three cells at each extremity of the trypanosome with a clear bridge of its active body visible between them.

A positive preparation may show every degree of red-cell adhesion, from a single cell to a complete "caddis" envelope; but the stronger the reaction the commoner are the "caddis forms."

In addition to red cells leucocytes are occasionally seen adhering, and in certain preparations, specified in the records that follow, definite platelet adhesion was seen either alone or simultaneously with the predominating red-cell type. Another phenomenon which we have learnt to regard as specific is the attachment of the parasites to the glass—sometimes quite obviously anchored by a platelet.

(*f*) *Strength of trypanosome blood.*

The number of trypanosomes required for a proper performance of this test is, according to our experience, not a very important factor. In this respect also our experience differs from that of the workers we have cited. Johnson and Lester, for instance, worked with a *T. gambiense* showing as many as thirty trypanosomes to a 1/6th field: only a few of our guinea-pigs reached this standard. Naturally a scanty infection demands more time, but, as already noted, the scattered parasites in a positive test easily catch the eye, standing out in relief as though marked with little waving flags.

On the other hand, mere numbers are not always an advantage. With our strains we have found that bloods heavily infected with either *T. gambiense* or *T. rhodesiense* are often very irregular in their response. Johnson and Lester believe that the adhesin may be exhausted when the parasites are very numerous, so that the reaction is incomplete. Leupold, on the other hand, ascribes these partial reactions to imperfect correspondence between the adhesin and the trypanosome—in other words, to incomplete serological identity. This latter interpretation agrees with our own experience—the more virulent the strain the less stable serologically.

(*g*) *Description of symbols employed.*

$-$ = less than 10 per cent. adherent.
tr = 10–19 per cent. adherent.
$+$ = 20–49 per cent. adherent.
$++$ = 50–59 per cent. adherent.
$+++$ = 60–89 per cent. adherent.
$+++\pm$ = 90 per cent. and upwards, adherent, *i.e.* a few clear trypanosomes.
$++++$ = 100 per cent. adherent, *i.e.* no free trypanosomes.

We have not adopted Brussin and Beletzky's (1925) method of recording results, followed both by Brown and Davis (1927) and by Johnson and Lester, because we do not find its lower limits sufficiently definite. The important

question of non-specific adhesion has not, we consider, received enough attention in any of these papers. That Johnson and Lester have encountered this phenomenon is strongly suggested by their exclusion of +---- results from their analysis of tests performed with cases of human trypanosomiasis.

(*h*) *Controls.*

As a rule in any daily group of tests there will be one or two negative preparations that will serve as controls. Failing these, it is advisable to put up a preparation of the tryp. blood alone. Red-cell adhesion is sometimes observed in fresh coverslip preparations of an infected animal's blood but disappears when the citrate is added. With *T. congolense* this tendency is, however, more marked, and in the tests involving this trypanosome an emulsion control is advisable.

In addition to emulsion controls we have tested a number of normal animals against sensitive trypanosomes. The object of these general controls is to see whether the red-cell adhesion reaction is ever given by the blood of animals that have never suffered from trypanosomiasis.

The details of the human blood controls are given in Part II. In connection with Part I ten normal monkeys were tested, each against two or more sensitive trypanosomes. All were negative according to our system of classification of results: occasionally 1 or 2 and once 4 per cent. of "caddis forms" were seen. A clean monkey and two healthy natives received appropriate doses of Bayer and their blood was subsequently tested against sensitive trypanosomes, in each case with negative results.

(*i*) *Nomenclature of strains used.*

And now a few words about the nomenclature of our strains. Without attempting here to discuss the vexed question of the relationship of the fly-borne polymorphic trypanosomes to one another, we may state, in passing, that in our opinion the diagnosis of trypanosomes from natives who have been in contact with two species of tsetse, one of which is *G. palpalis* and the other a game-tsetse, is not a simple and straightforward matter.

It will be seen below that several of the *T. gambiense* adhesin bloods responded very definitely with both *T. rhodesiense* and the Damba strain. We shall discuss this phenomenon superficially later on, but it is necessary at this juncture to make quite clear what is meant by *T. gambiense* and by *T. rhodesiense* in this report.

In European laboratories the classic names *T. gambiense* and *T. brucei* are borne by strains which are utterly different from the originals to which these names were first applied. These strains have, some of them, been kept up for many years by syringe-passage from rodent to rodent, and it is with such strains that the great bulk of experimental work on the adhesion phenomenon has been performed. As already stated, *all* the strains dealt with below have recently passed cyclically through tsetse, although some of them have been

maintained at the Laboratory since early in 1926. Our short experience of the adhesion test has revealed certain apparent affinities—if we may so interpret our unexpected results—between trypanosomes which are, to say the least, vastly further apart zoologically than are the "passage" and "relapse" variants of one and the same strain. It is essential, therefore, to call attention to a point which, but for this strange overlapping between "species" shown by our adhesion tests, would not have appealed to us as possessing any importance, namely that one group of our *T. gambiense* strains, "W," "Y" and "European," comes from the West Nile area, whence come also the puzzling strains Okero, Dom and "E" discussed in Part II, Section 1.

Scattered through the West Nile area there is *G. morsitans*: *G. palpalis* also occurs almost everywhere where there is suitable water and shade, that is, along most of the streams in the area.

Strains "W," "Y" and "European" have, however, always comported themselves in a manner absolutely typical of *T. gambiense*, and there is no reason, other than that supplied by the adhesion test, to question their claim to that title. And yet it is strain "Y" that has produced the most striking examples of overlapping with *T. rhodesiense* trypanosomes.

The other group of *T. gambiense* strains hailed from the Lake Victoria infected areas, where there is no *G. morsitans*: save in respect of the adhesion test, this group is above suspicion, "typical *T. gambiense*."

Our *T. rhodesiense* strain offers no difficulty apart from the fact that since its first isolation from a patient in May, 1927, it has been in contact, at the Entebbe Laboratory, with *G. palpalis* and not with its normal vector. It is possible that the strain has in consequence undergone some subtle modification, too obscure to be noticed by the tests at our disposal. To all appearances the general attributes of this strain are still very much the same as those of the early passages. It was recovered from a native at Ikoma near Mwanza in Tanganyika Territory, where *T. rhodesiense* appeared some years ago in epidemic form.

Strains Okero, Dominiko and Eli (referred to below as "Okero," "Dom" and "E" respectively) have not yet been worked out. Okero and Dominiko had received treatment with trypanarsol; Eli had undergone a full course of tryparsamide. At present all that can be said is that all three, in man, are completely resistant to tryparsamide; that all reacted immediately to Bayer 205; and that monkeys and guinea-pigs infected with strain Okero show large numbers of trypanosomes in their peripheral blood early in the disease. In all these respects this last strain differs from typical *T. gambiense*. The occurrence and frequency of posterior-nuclear forms is being investigated. Strain Dominiko has not produced much effect on the single monkey hitherto infected, and strain Eli has only just been recovered from the patient.

These three strains illustrate the difficulties attending the recognition and naming of the polymorphic trypanosomes. An arsenic-resistant *T. gambiense* may either be something altogether new, or it may be a strain picked up by

tsetse from a case that has relapsed after treatment, and passed on unchanged to a new victim; or again it may be a *T. rhodesiense* in chastened mood. In an area such as the West Nile district of the Uganda Protectorate where a native population is in contact with the two principal insect vectors of human trypanosomiasis, and trypanosomiasis gambiensis has existed for years, it is difficult to detect the earliest incursions of the other and more mysterious form of the disease. We have, then, before us an interesting scientific and an important economic problem, in the investigation of which the adhesion test has played an instructive rôle.

We will now proceed to the experiments themselves.

The elaboration of the next section is necessary if we are to present a true picture of the astonishing variability of one or both of the two essential factors in the test—the adhesin blood and the tryp. blood. This is especially noticeable in the case of *T. rhodesiense*. In fact the revelation of this variability is the chief object of this contribution, and we are convinced that a realisation of the limitations of the test is a necessary step towards its proper application to the diagnosis of chronic trypanosome infections.

Part I deals with animal experiments with *T. gambiense*, *T. rhodesiense*, double infections of these two, the Damba trypanosome and *T. congolense*, each species being treated in a separate section beginning where necessary with a genealogical table giving the history of the strains.

Only animals actually tested for adhesins are numbered, the intervening passages being shown by letters. The *T. gambiense* section commences with an Index Table where the experimental animals are shown in numerical order with the date on which each was first infected. Reference can thus be made from this index to the corresponding genealogy for further detail.

As we have only one strain each of *T. rhodesiense* and Damba trypanosome no other indices are necessary.

Part II consists of two sections; the first is devoted to selected human cases that were studied at the Laboratory Hospital, including the three newly acquired strains Okero, Dominiko and Eli; the second to the application of the adhesion test to a number of cases of human trypanosomiasis in the West Nile area of the Uganda Protectorate.

(*j*) *General explanation of the tables that follow and the symbols used therein.*

Each animal has an experimental number, these numbers being printed in three different ways; ordinary type (613) indicates an infection with *T. gambiense*, italics (*619*) *T. rhodesiense*, large heavy type (**631**) the Damba strain, and small heavy type (**633**) strain Okero. The letter "d" in brackets after certain experimental numbers indicates a double infection with *T. gambiense* and *T. rhodesiense*: the letter "p" in brackets after a positive symbol indicates platelet-adhesion only; "p and c" = a mixture of platelet and red-blood-corpuscle adhesion: otherwise red-cell adhesion is implied.

In the Genealogical Tables II, VI, IV, VIII and X a single line means that the passage was effected by the inoculation of citrated blood; a double line shows a cyclical passage through laboratory-bred *G. palpalis*. In these tables only animals actually referred to in this paper are mentioned in full, the intervening passage being shown by letters: "M" = monkey, "Sh" = sheep, "Gt" = goat, "P" = guinea-pig.

In Tables III, V and VII, devoted respectively to *T. gambiense* and *T. rhodesiense* infections, where trypanocidal drugs were administered the details are given in brackets after the animal's experimental number.

Table V deals with two *T. gambiense* animals that were superinfected with *T. rhodesiense*, the date of the second infection and other relevant information being given under the animal's experimental number.

Table III, comprising Sections 1–17, shows the response of selected animals infected with *T. gambiense* to different strains of trypanosomes. Table VII is its counterpart for *T. rhodesiense*. These two tables are complementary to Tables IV and VIII, which illustrate the behaviour of selected strains of these two trypanosomes when exposed from day to day to different adhesin bloods. Tables III and VII are thus arranged to show the range, sensitiveness and at the same time the inconstancy of the adhesion reaction in a series of selected positive bloods: the variability of the behaviour of the trypanosomes in one and the same animal on different days is, on the other hand, better shown by Tables IV and VIII.

Table X treats of the Damba strain, showing its reactions when exposed to blood from well-established infections of *T. gambiense*, *T. rhodesiense*, the Damba strain, and strain Okero (cf. Part II).

II.

PART I. EXPERIMENTS WITH *T. GAMBIENSE*, *T. RHODESIENSE*, THE DAMBA TRYPANOSOME AND *T. CONGOLENSE* IN LABORATORY ANIMALS.

Section 1. T. gambiense *animals.*

Table I.

Index of strains used.

M 422	Strain	V*	M 601	Strain	European
427	"	V*	606	"	"
451	"	V*	607	"	"
479	"	Diri	612	"	"
487	"	Yowana	613	"	"
488	"	Simoni	614	"	Yembuga
501	"	European	622	"	"
513	"	Waiga	623	"	"
516	"	Yembuga	624	"	V
544	"	"	625	"	V
548	"	XI*	628	"	V
556	"	Yembuga	632	"	European
557	"	"	P 16 a; 17 d, da, e, f, g, h, j; 25 a, b, c, d	"	Yembuga
597	"	"			
598	"	"			

* These strains are identical with those of the same designation in the *Final Report of the League of Nations International Commission on Human Trypanosomiasis.*

Table II. *Genealogies of these strains.*

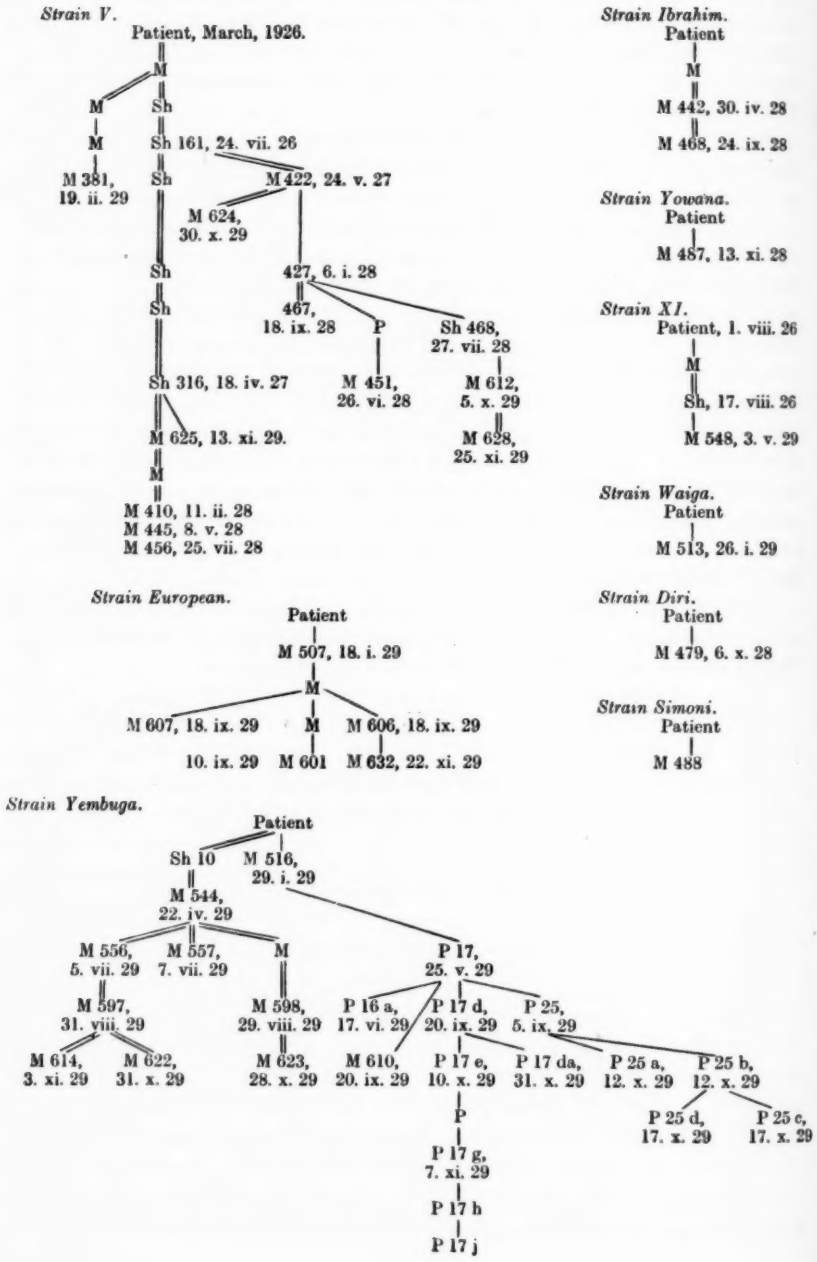

Table III.

Selected T. gambiense *animals tested against different trypanosomes.*

(Sections 1–17.)

Animal	Date	Tryp.	Adhesion
Section 1.			
M 422	Oct. 9	P 25	+ + + +
	„ 9	P 17	+ + + ±
	„ 11	**P 30**	–
	„ 11	P 25	+ + +
	„ 11	P 17	+ + +
	„ 12	P 25	+ + + ±
	„ 12	P 17 d	+ + +
	„ 12	P 17	+ +
	„ 12	P 8	+ + + +
	„ 12	P *34*	–
Section 2.			
M 427	Oct. 9	P 25	+ + + ±
	„ 9	P 17	+ + +
	„ 25	613	–
	Nov. 8	623	+ + + +
	„ 15	P 17 g	+ + +
	„ 15	P 17 da	+ 33%
	„ 18	*619*	+ +
	„ 28	P 25	–
	„ 28	624	+ + + +
	Dec. 6	**633**	– 8%
	„ 6	P 25	tr 15%
Section 3.			
M 442	Oct. 16	P 17	+ + + ±
	„ 16	P 25	+ + + +
	„ 18	P *34*	–
	Nov. 21	*621*	–
	„ 29	624	+ + + ±
Section 4.			
M 451	Oct. 14	P 17	+ + +
	„ 14	P 25	+ + +
	„ 26	P 17 e	+ + + +
	„ 28	P 25	+ +
	Nov. 6	P 25	+ + + ±
	„ 15	P 17 g	+ + + + (p & c)
	„ 15	P 17 da	+ + + + (p & c)
	„ 18	*619*	–
	„ 28	P 25	–
	„ 28	624	+ + +
	Dec. 3	**633**	–
	„ 6	**633**, P 25	–
	„ 11	**631**	–
	„ 13	P 25, P 17 h	–
	„ 13	P 17 g	+ 33%
	„ 13	P 17 j	+ + +
	„ 17	*638*	–
	„ 17	628	tr 18%
	„ 19	**642, 643**	–
	„ 19	P 17 h, 25	+ 40%
	„ 19	P 17 g	+ +
	„ 20	632	+ 25%
	„ 20	**P 30**	+ 25%
	„ 23	*638*, **631**	–
	„ 30	**654, 653**	–
Section 5.			
M 467	Oct. 10	P 25	–
	„ 10	P 17	–
	Nov. 15	P 17 da	–
	„ 15	P 17 g	+ + + (p & c)
	„ 18	*619*	tr 17%

Animal	Date	Tryp.	Adhesion
Section 6.			
M 479	Oct. 9	P 25	–
	„ 9	P 17	–
	„ 23	P 25	–
	Nov. 15	P 17 g	+ + + +
	„ 15	P 17 da	–
	„ 18	*619*	– 8%
Section 7.			
M 487	Oct. 10	P 17	+ + +
	„ 10	P 25	+ + +
	Nov. 10	*619*	–
	„ 28	P 25	–
	„ 28	624	+ + + +
	Dec. 13	P 25	–
	„ 13	P 17 g	+ 33%
	„ 19	**642, 643**	–
	„ 19	P 17 h	+ + +
	„ 19	P 25, P 17 g	+ + + +
	„ 20	632	+ + + +
	„ 20	**P 30**	–
	„ 23	*638*, **631**	–
	„ 30	**654**	–
	„ 30	**653**	+ 33%
Section 8.			
M 488	Oct. 10	P 25	+ + +
	„ 10	P 17	+ + +
	„ 11	**P 30**	–
	„ 11	P 25	+ + +
	„ 11	P 17	+ + + ±
	„ 16	P 25	+ + + ±
	„ 25	613	+ 20%
	Dec. 19	**642, 643**	–
	„ 19	P 25	–
	„ 19	P 17 g	+ +
	„ 19	P 17 h	+ + + +
	„ 20	632, **P 30**	–
	„ 23	*638*, **631**	–
	„ 30	**654, 653**	–
Section 9.			
M 507	Oct. 9	P 25	+ + + +
	„ 9	P 17	+ + + +
	„ 9	P *33*	–
	„ 24	P 17	+ + + ±
	„ 24	P 25	+ + +
	„ 24	P *33*, P *34*	–
	„ 27	P 17 e	+ + + +
	„ 27	613	+ 38%
	„ 26	P 17 c	+ + + ±
	Nov. 3	*617*	–
	„ 4	P 17 d	+ + + +
	„ 6	P 25	+ + + +
	„ 15	P 17 g	+ + + +
	„ 15	P 17 da	+ + + +
	„ 15	*619*	+ 20%
	„ 19	*621*	–
	„ 19	P 25	–
	„ 20	P 25	–
	„ 20	624	+ + + +
	Dec. 2	**P 30**	–
	„ 2	P 25	–
	„ 3	**631, 633**	–

Table III (*cont.*).

Animal	Date	Tryp.	Adhesion
Section 9.	Dec. 4	614, **633**	–
M 507	„ 6	P 25	+ 30%
(*cont.*)	„ 6	**633**	–
Section 10.			
M 513	Oct. 10	P 17 g	+ +
	„ 10	P 25	+ + +
	Nov. 8	P 25, 624	+ + + +
	Dec. 4	614	+ + + +
	„ 5	**633**	– 9%
	„ 6	**633**	–
	„ 6	P 25	+ + + +
	„ 7	**631**, 633	–
	„ 11	*635*	tr 10%
	„ 11	**631**	+ + + ±
	„ 16	**631**	– 8%
	„ 16	**636**	+ + + +
	„ 16	P **30**	–
	„ 20	632	+ + + ±
	„ 20	P **30**	–
	„ 23	*638*, **631**	–
	„ 24	628	+ + + +
	„ 24	**636**	tr 13%
Section 11.			
M 556	Oct. 14	P 17	–
	„ 14	P 25	–
	„ 25	P 17	–
	„ 25	P 25	–
	Nov. 11	606	–
	„ 13	606	–
	„ 14	P 25	–
	„ 15	P 17 g	+ + + +
	„ 15	P 17 da	–
	„ 28	P 25	–
	„ 28	624	–
Section 12.			
M 598*	Oct. 14	P 17	+ + +
	„ 14	P 25	+ + + ±
	„ 23	P 25	+ + + +
	„ 23	*609*, P 17 d	–
	„ 25	613	–
	„ 28	P 17 e	–
	„ 28	P 25	+ + + ±
	Nov. 3	*617*	– 8%
	„ 4	P 17 d, P 17 e	–
	„ 6	P 17 e	tr 15%
	„ 6	P 17 d	–
	„ 6	P 25	+ 30%
	„ 17	*619*	–
	„ 29	624	+ + +
Section 13.			
M 601	Oct. 14	P 17	+ 40%
	„ 14	P 25	+ + + +
	„ 25	P 17	+ 35%
	„ 25	P 25	+ + + ±
	Nov. 6	P 25	+ + + ±
	„ 18	P 17 da	+ + + +
	„ 18	P 17 g	+ + + +
	„ 28	P 25	–
	„ 28	624	+ + +
	Dec. 3	**633**	–
	„ 6	P 25	+ + + ±
	„ 6	P **30**	–
	„ 11	**631**	–
Section 14.			
M 612	Oct. 16	P 25, P 17	–
	„ 22	*609*, P *6* q, P *6* r	–
	„ 25	P 17, P 25	–

Animal	Date	Tryp.	Adhesion
Section 14.	Nov. 6	P 25	+ + + +
M 612	„ 6	P 17 e, 17 d	–
(*cont.*)	„ 27	P 17 g	+ + + (p & e)
	„ 27	*619*, *620*	–
	„ 27	*621*	+ 40%
	„ 28	624	+ + +
	Dec. 6	P **30**	–
	„ 6	P 25	+ + + +
	„ 7	**633**	–
	„ 7	**631**	tr 17%
	„ 9	614, **633**, *619*, *620*	–
	„ 9	P 17 g	+ 25%
	„ 10	**631**	+ + + +
	„ 10	P 17 g	+ +
	„ 10	P 17 h	+ + +
	„ 10	*619*	–
	„ 16	**631**	tr 18%
	„ 16	**636**	+ 40%
	„ 16	P **30**	–
	„ 23	*638*, **631**	–

Date	Tryp.	Adhesion 544	Adhesion 516
Section 15.			
Oct. 9	P 17	+ + +	+ + + +
„ 9	P 25	+ + +	+ + + ±
„ 9	P 33, *6*	–	–
„ 25	613	tr 18%	+ 30%
„ 26	P 17 e	+ + + +	+ + + +
„ 31	17 d	+ + + +	.
Nov. 4	P 17 d	+ + + +	+ + + ±
„ 4	P 17 e	.	+ + + +
„ 6	P 17 e, 25	+ + + +	.
„ 7	606	+ + + +	+ + + ±
„ 8	623	+ + + +	.
„ 11	606	+ + + +	.
„ 14	*619*	–	.
„ 14	P 25	+ + + ±	.
„ 15	P 17 da	+ + + +	+ + + +
„ 15	P 17 g	+ + + ±	+ + + +
„ 17	*619*	+ + +	.
„ 19	*621*	+ 40%	+ 40%
„ 19	P 25	+ + + ±	.
„ 20	*621*	–	tr 10%
„ 22	*619*	+ + + ±	+ 30%
„ 22	*620*	+ 35%	–
„ 22	*621*	–	–
„ 28	P 25	+ + + ±	+ + + +
„ 28	624	+ + + +	+ + + ±
Dec. 2	P **30**	–	.
„ 2	P 25	+ + + +	.
„ 3	**633**	–	.
„ 4	**633**	–	–
„ 4	614	+ + + ±	+ + +
„ 4	**631**	+ 26%	–
„ 5	*620*, **633**	–	–
„ 6	633, P **30**	–	–
„ 6	P 25	+ + + ±	+ + + +
„ 7	**631**, **633**	–	–
„ 9	*619*	+ + + +	.
„ 9	*620*, **633**	–	.
„ 9	614	+ +	.
„ 9	P 17 g	+ 30%	.
„ 10	**631**	–	.
„ 10	P 17 g	tr 13%	.

* On 9. x. 29 received atoxyl 0·03 grm. which sterilised its blood.

Table III (*cont.*).

Date	Tryp.	Adhesion 544	Adhesion 516
Section 15. (*cont.*)			
Dec. 10	P 17 h	+ + + +	
„ 10	*619*	+ + + +	•
„ 11	*635*	tr 10%	+ 25%
„ 11	**631**	+ + + ±	+ + + +
„ 16	**631**	+ 25%	+ 25%
„ 16	P**30**	+ +	+ 45%
„ 16	**636**	+ + + ±	–
„ 17	628, *638*	+ 20% 20%	+ 20% 40%
„ 18	P 17 g, 17 h	.	+ + + ±
„ 18	624	.	+ + + +
„ 19	**642**	tr 15%	–
„ 19	**643**	–	–
„ 20	632	+ + +	+ 33%
„ 20	P**30**	–	–
„ .		+ 25%	+ 23%
„ 23	**631**	–	–
„ 24	628	+ + + +	+ + + +
„ 24	**636**	+ 25%	–
„ 30	**654**	–	–
„ 30	**653**	tr 14%	+ 30%
Jan. 2	646, **647**	.	–
„ 7	**651, 653**	–	.
„ 7	**652**	tr 12%	.
„ 7	*656*	+ + + ±	.
„ 8	**651**	–	.
„ 8	**656**	+ + + ±	.
„ 9	646	+ + + +	+ + + +

Animal	Date	Tryp.	Adhesion
Section 16.			
P 16 a (g)	Oct. 17	P 17	+ + (p)
	„ 17	P 25	+ + + + (p)
	„ 27	P 17 e	+ + + + (p)
	„ 27	613	–
	„ 28	P 17 e	+ + + ± (p)
	„ 28	P *34*	–
	„ 28	P 17 d	+ + + ± (p)
	Nov. 11	P 17 d	+ + + + (p)
	Jan. 6	628	+ + + (red-cell adhesion)
Section 17.			
P 17 c (g)	Sept. 6	P 17 b	–
	„ 28	P 25	+ +
	Oct. 1	P 8	–
	„ 1	P 17	–
	„ 1	P 17 d	+ + + (p)
	„ 12	P 8	–
	„ 12	P *34*	–
	„ 12	P 17	–
	„ 12	P 17 d	–
	„ 12	P 25	–
	„ 15	P 25	–
	„ 15	P 17	–
	„ 27	613	–
	„ 27	P 17 e	–

Table IV.

Trypanosomes from consecutive guinea-pig-passages—17, 17 d, e, da, g, h, j, *and guinea-pigs* 25 (cf. Table II) *exposed to different positive adhesin bloods.*

Date	Adhesin blood	Tryp. blood 25	Tryp. blood 17
Sept. 23	*578*	–	–
„ 25	P 17 c	+ +	–
„ 28	P 17 c	+ +	–
Oct. 9	544	+ + + +	+ + + ±
„ 9	427	+ + + ±	+ + +
„ 9	422	+ + + +	+ + + ±
„ 9	516	+ + + ±	+ + + +
„ 9	507	+ + + +	+ + + +
„ 10	381	+ + + ±	–
„ 10	513	+ + +	+ +
„ 10	487	+ + + ±	+ + + ±
„ 10	488	+ + +	+ + +
„ 11	488	+ + +	+ + + ±
„ 11	422	+ + +	+ + +
„ 12	422	+ + + ±	+ + (p & c)
„ 14	597	+ + + +	+ + +
„ 14	598	+ + + ±	+ + +
„ 14	557	+ + + ±	+ +
„ 14	607	+ +	+ +
„ 14	451	+ + +	+ + +
„ 14	601	+ + + +	+
„ 15	490	+ + +	tr
„ 15	610	+ + + +	+ + + ±
„ 15	Bl.	+ +	+ 20%
„ 16	468	+ +	+ + + ±
„ 16	442	+ + + +	+ + + ±
„ 16	410	+ + +	+ + + ±

Table IV (*cont.*).

Date	Adhesin blood	Tryp. blood 25	Tryp. blood 17	Tryp. blood 17 d	Tryp. blood 17 e
Oct. 16	548	+ + + ±	+ + + ±		
„ 17	P 16 a	+ + + + (p)	+ + (p)		
„ 19	557	+ + + +	+	+ 20%	.
„ 21	Musa	+ 30%	+ +	−	.
„ 23	557	+ + + ±	.	−	.
„ 23	598	+ + + +	.	.	.
„ 24	Musa	tr 10%	+ + + ±	.	.
„ 24	507	+ + +	+ + + ±	.	.
„ 25	610	+ 33%	+ +	.	.
„ 25	612, 556	−	−	.	.
„ 25	601	+ + +	+ 35%	.	.
„ 27	507	−	−	−	+ + + +
„ 28	P 16 a	.	.	+ + + ± (p)	+ + + ± (p)
„ 28	Bl.	.	.	tr	tr 10%
„ 28	544	.	.	+ + + +	+ + + +
Nov. 4	610	.	.	+ + + +	+ + + +
„ 4	516	.	.	+ + + + (p & c)	+ + + + (p & c)
„ 6	598	+ 30%	.	.	tr 15%
„ 6	544, 507	+ + + +	.	+ + + +	+ + + +
„ 6	612	+ + + +	.	−	−
„ 6	Musa	+ 30%	.	+ 30%	+ 46%
„ 11	Okero	−	.	+ +	+ + +

Date	Adhesin blood	Tryp. blood 17 da	Tryp. blood 17 g	Tryp. blood 17 h	Tryp. blood 17 j
Nov. 15	601	+ + + +	+ + + +	+ + +	.
„ 15	451	+ + + + (p & c)	+ + + + (p & c)	+ + + +	.
„ 15	544	+ + + +	+ + +	−	.
„ 15	516	+ + + +	+ + + +	+ 34%	+ +
„ 15	427	+ 33%	+ + +	− 9%	+ + +
„ 15	507	+ + +	+ + + +	.	.
„ 15	556	−	+ + + +	−	+ + +
„ 15	467	−	+ + + (p & c)	−	+ + +
„ 15	479	−	+ + + ± (p & c)	+ + + ±	+ + +
„ 15	Okero	+ + + ±	+ (p & c) 45%	+ + + ±	.
„ 15	Dom	−	+ +	+ + +	.
Dec. 10	612	.	+ +	+ + + +	.
„ 10	544	.	tr 13%	−	.
„ 10	624, **631**	.	−	+ 34%	+ +
„ 12	381	.	+ + + ±	− 9%	+ + +
„ 12	451	.	+ 33%	.	.
„ 12	487	.	+ 33%	−	+ + +
„ 12	456	.	+ + + ±	−	+ + +
„ 12	Okero	.	−	+ + + ±	+ + +
„ 12	607	.	.	+ + + ±	.
„ 18	516	.	+ + + ±	+ + +	.
„ 18	607	.	+ 40%	+ + +	+ + + +
„ 19	487	.	+ + + +	+ + + +	.
„ 19	488	.	+ +	+ 40%	.
„ 19	451	.	+ +	+ +	.
„ 19	Dom	.	+ +		

Table V.

Tests with two T. gambiense *Monkeys superinfected with* T. rhodesiense.

M 557

On 9. x. 29 received 0·03 grm. atoxyl which sterilised its blood from *T. gambiense*.

On 21. x. 29 was inoculated with *T. rhodesiense* from guinea-pig 33.

On 6. xi. 29 received 0·1 grm. Bayer which sterilised its blood from *T. rhodesiense*.

Date	Tryp. blood	Adhesion
Oct. 14	P 25	+ + + ±
„ 14	P 17	+ +
„ 16	P 25	+ + + +
„ 19	P *34*	–
„ 19	P 25	+ + + +
„ 19	P *33*	–
„ 19	P 25	+ + + +
„ 23	P 17 d	–
„ 23	P *6* q	–
„ 23	P 34	–
„ 23	*609*	+ +
„ 23	P 25	+ + + ±
„ 24	P *33*	–
„ 25	613	–
Nov. 14	*619*	+ 40%
„ 14	P 25	+ + +
„ 18	*619*	–
„ 20	*621*	+ + + ±
„ 21	*619*	–
„ 21	*621*	+ + + +
„ 22	*619, 620*	–
„ 22	*621*	+ + + +
„ 26	*620*	–
„ 26	*621*	+ + + +
„ 26	P 17 g	–
„ 27	*620*	–
„ 27	*621*	+ + + +
„ 27	P 17 g	–
„ 27	*619*	–
„ 29	624	+ + + ±
Dec. 1	*619*	–
„ 4	**633**	–
„ 4	614	– 8%
„ 5	**633**	tr 12%
„ 7	**631, 633**	–
„ 11	*635*, **631**	–
„ 20	632	–
„ 23	*638*	+ +
„ 23	**631**	–

M 597

On 9. x. 29 received atoxyl 0·03 grm. which sterilised its blood from *T. gambiense*.

On 27. x. 29 was superinfected by flies from monkey 603 (*T. rhodesiense*).

On 6. xi. 29 received a sterilising dose (0·1 grm.) of Bayer.

Date	Tryp. blood	Adhesion
Oct. 14	P 17	+ + +
„ 14	P 25	+ + + +
„ 19	P *33*	–
„ 19	P *34*	–
„ 25	613	–
Nov. 4	P 17 d	+ 19%
„ 14	*619*	–
„ 14	P 25	+ 47%
„ 17	*619*	–
„ 20	*621*	–
„ 21	*619*	–
„ 21	*621*	–
„ 27	P 17 g	–
„ 29	624	+ + +
Dec. 7	**631, 633**	–
„ 11	*635*	+ + + ±
„ 11	**631**	–
„ 17	*638*	+ 40%
„ 17	*635*	+ + + ±
„ 17	628	–

Remarks on the T. gambiense *tables.*

Of special interest in Table III are the following points:

(*a*) A negative reaction possesses no diagnostic significance.

(*b*) The length of time the reaction may persist in an untreated animal as shown by monkey 422 (two years and four and a half months); monkeys 427, 442, 451, 487, 488 all gave strong reactions more than a year after their original infection. None of these animals was ever treated with drugs. Brussin and

Beletzky mention that Rieckenberg's animals retained their reacting power for over two and a half months.

(*c*) The curious fact that on 15. xi. 29 trypanosomes from guinea-pig 17 g evoked a strong response from three bloods which had failed on several occasions to respond with proved sensitive tryp. blood, *i.e.* 467, 479 and 556.

(*d*) The readiness with which *T. gambiense*, when first it appears in the peripheral blood of a monkey, calls forth a positive reaction; *vide* the strong reaction obtained with the trypanosomes from monkeys 624, 628 and 632.

(*e*) The occasional strong response of monkeys 513, 612, 544 and 516 to the Damba trypanosome.

(*f*) The strong response given at times by monkey 544 with the *T. rhodesiense* trypanosomes from monkey 619, and to a less extent with monkey 621. Monkey 516 also responded occasionally to the same two tryp. bloods; and once monkey 427, infected with a Lake Victoria strain, gave a 50 per cent. reaction with *T. rhodesiense*.

(*g*) The repeated failure of any of these monkeys to respond to the trypanosomes from Okero and Dominiko.

(*h*) In Table IV note the difference between the behaviour of guinea-pigs 17 da and 17 g.

(*k*) In Table V both monkeys 557 and 597 after superinfection with *T. rhodesiense* and the injection of Bayer 205 responded strongly to *T. rhodesiense* and also to *T. gambiense* (624).

(*l*) Sections 16 and 17 of Table III are of interest, being the only two guinea-pig bloods to give definite positive reactions. Guinea-pig 16 a was treated with atoxyl as follows:

Date	Atoxyl dose grm.
11. vii. 29	0·002
12. vii. 29	0·003
22. vii. 29	0·004
31. vii. 29	0·005
12. viii. 29	0·005
31. viii. 29	0·006

This treatment sterilised the animal of trypanosomes.

Guinea-pig 17 c received a single sterilising dose of Bayer 205 on 10. ix. 29.

Of sixteen other guinea-pigs infected with *T. gambiense* and tested for adhesins, fourteen were sterilised with atoxyl and two were left untreated. They were all tested with sensitive trypanosomes from guinea-pigs 25 and 17; the majority gave completely negative results, two a feeble reaction in which only platelets adhered.

It will be seen that in all the positive reactions given by 16 a and 17 c *only platelets adhered to the trypanosomes*, save only when 16 a blood was tested with tryp. blood from monkey 628, when 80 per cent. red-cell adhesion resulted.

We shall discuss this point later on. Evidently the guinea-pig, though in some ways a suitable animal from which to draw trypanosomes for the adhesion test, cannot be relied upon to supply a good adhesin blood.

Section 2. T. rhodesiense *animals.*

Table VI.

Genealogy of strains.

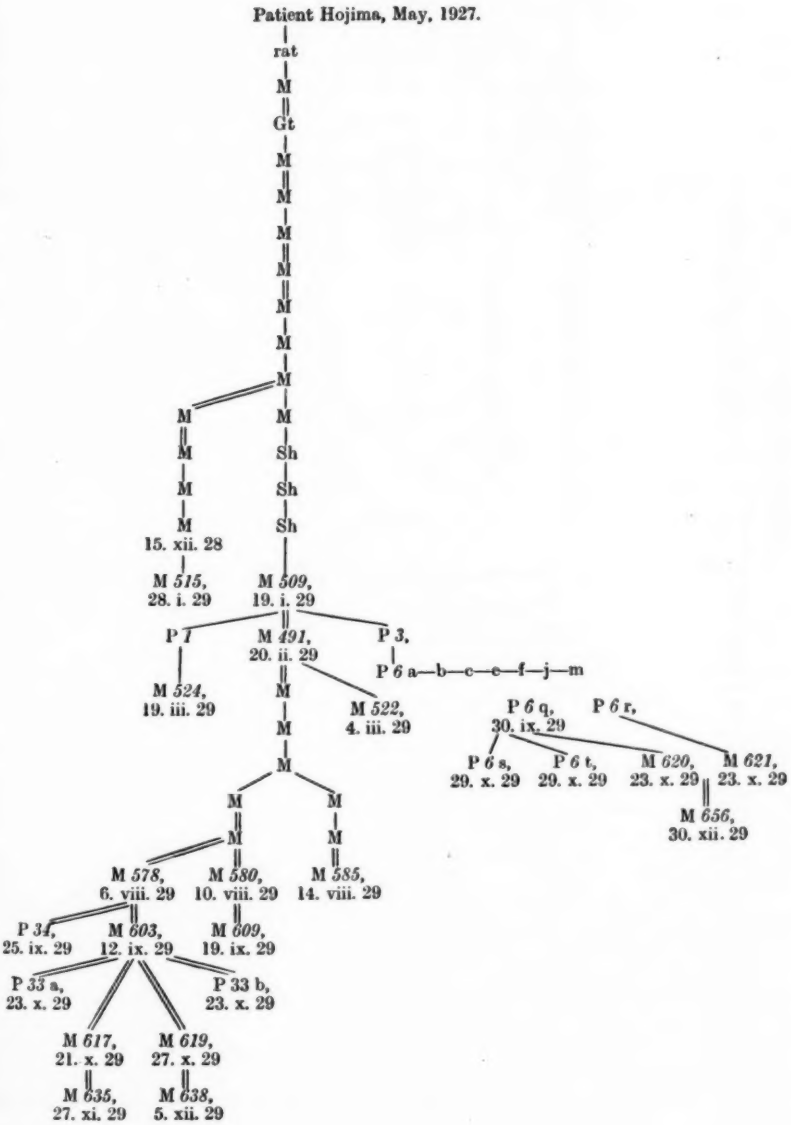

Table VII.

Selected T. rhodesiense *monkeys Bayerised and tested against different trypanosomes.*

(Section 1.)

Date	Tryp blood	Adhesin blood: *M 524* (0·025 Bayer, 4. iv. 29; reinfected, 27. vi. 29; 0·025 Bayer, 17. vii. 29)	*M 578* (0·025 Bayer, 4. ix. 29)	*M 580* (0·025 Bayer, 6. ix. 29)	*M 522** (0·025 Bayer, 16. iv. 29 0·025 Bayer, 15. v. 29)	*M 585* (0·025 Bayer, 31. viii. 29)
Sept. 28	*609*	.	+ + + ±	.	+ + + +	.
„ 28	*603*	.	+ + + +	.	+ + + +	.
Oct. 1	*609*	+	+ + + +	.	+ + +	+ + + +
„ 1	P *33*	.	−	.	.	−
„ 4	P 25	.	−	−	.	.
„ 4	*609*	.	+ + + ±	+ + + ±	.	.
„ 11	P **30**	.	−	−	.	.
„ 18	P *33*	.	−	−	−	−
„ 18	*609*	.	+ + + ±	+ + +	+ 30%	−
„ 19	P *34*	.	.	+ + +	+ 20%	−
„ 19	P *33*	.	.	−	−	.
„ 21	*609*	.	+ 20%	+ + + ±	.	.
„ 22	P *6* r	−	−	−	−	−
„ 22	P *6* q	+ 28%	−	−	+ 30%	tr 11%
„ 24	P *34*	.	−	+ 40%	.	.
„ 29	P *23*, P *34*	.	.	−	.	.
„ 30	P *34*	+ + + ±	.	+ + + ±	.	−
„ 31	P *23* a	.	tr 13%	+ + + +	.	.
„ 31	P *23*	−	−	−		.
Nov. 1	P *23* a	−	.	+ + + +	.	.
„ 3	*617*	+ + +	+ + +	+ + + +	.	−
„ 4	*597*	+ + + ±	+ + + ±	+ + + +	+ + + ±	+ + + ±
„ 4	*557*	.	.	+ + +	+ 40%	.
„ 4	*617*	.	+ + +	+ + + +	+ + + ±	− 7%
„ 4	*619*	.	.	+ + +	+ + + +	.
„ 6	*619*	+ + + ±	−	.	.	.
„ 6	*620*	+ +	− 8%	+ + +	.	− 8%
„ 9	*619*	−	−	−	+ + +	−
„ 9	*620*	−	.	−	.	.
„ 10	*619*	− 6%	tr 15%	−	+ +	.
„ 10	*620*	−	−	−	+ 30%	.
„ 18	*619*	−	−	−	.	.
„ 19	*621*	.	+ + +	−	− 8%	.
„ 21	*619*, *621*	.	−	−	.	.
„ 22	*619*, *620*, *621*	−	−	−	−	.
„ 27	P 17 g	−	−	−	−	.
„ 27	*621*	.	+ + +	.	.	.
„ 28	P 25, 624	−	−	−	−	−
Dec. 2	P **30**	−	−	−	.	.
„ 3	**633**	.	−	−	.	.
„ 4	**633**, 614	.	−	−	.	−
„ 4	**631**	.	−	−	.	.
„ 7	**631**, 633	.	−	−	.	.
„ 11	*635*	.	tr 12%	+ 30%	tr 12%	−
„ 11	**631**	.	−	−	−	−
„ 20	632	.	−	−	.	−
„ 23	*638*	.	−	tr 10%	.	−

* Monkey 522 relapsed after its first dose of Bayer which was not administered until the forty-third day of its infection.

Table VII (*cont.*). Section 2.

M 515

28. i. 29 Originally infected
20. ii. 29 Bayer 0·003 grm.
25. ii. 29 „ 0·003 „
27. ii. 29 „ 0·01 „

Date	Tryp. blood	Adhesion
Oct. 1	*609*	+ + +
„ 4	P 25	–
„ 4	*609*	+ + +
„ 18	P *33*	–
„ 18	*609*	+ + +
„ 22	P 6 q	+ 26%
„ 22	P *6* r	–
„ 30	P *34*	+ + +
Nov. 4	*617*	+ + + +
„ 4	597	+ + + +
„ 21	*619*	+ + + ±
„ 21	*621*	+ + + ±
„ 22	*620*	+ 40%
„ 22	*619*	+ + +
„ 22	*621*	+ + + ±
„ 26	*620*	+ 20%
„ 26	*621*	+ 33%
„ 26	P 17 g	+ 40%
„ 27	*620*	+ 25%
„ 27	*621*	+ + +
„ 27	P 17 g	+ + +
„ 27	*619*	tr 12%
„ 28	P 25	–
„ 28	624	tr 10%
Dec. 1	*619*	–
„ 1	**P30**	–
„ 1	P 25	–
„ 2	**633**	–

M 515

2. iii. 29 Bayer 0·02 grm.
5. iii. 29 „ 0·03 „
4. xi. 29 Superinfected
19. xii. 29 Bayer 0·1 grm.

Date	Tryp. blood	Adhesion
Dec. 4	**633**	+ 26%
„ 4	614	+ + + +
„ 4	**631**	tr 15%
„ 5	**633**	+ +
„ 5	*620*	–
„ 7	**631**	–
„ 7	**633**	+ 25%
„ 11	*635*	+ + +
„ 11	**631**	+ +
„ 16	**631, 636**	–
„ 16	P**30**	+ 30%
„ 17	*638*	+ + + +
„ 17	628	–
„ 17	*635*	+ + + ±
„ 20	632	+ +
„ 23	*638*	+ + + +
„ 23	**631**	–
„ 24	628	+ + + ±
„ 24	**636**	+ +
„ 30	**654**	+ 20%
„ 30	**653**	+ 33%
„ 2	**647**	–
Jan. 7	**651, 653**	+ 33%
„ 7	656	+ + + +
„ 8	**651**	–
„ 8	656	–
„ 9	646	+ 25%

Table VIII. *Three selected nearly related passages of* T. rhodesiense *exposed to different adhesin bloods.*

Date	Adhesin blood	Tryp. blood *619*	Tryp. blood *620*	Tryp. blood *621*
Nov. 6	*578*	–	– 8%	.
„ 6	*524*	+ + + ±	+ +	
„ 9	*580, 524*	–	–	.
„ 9	*522*	+ + +	+ 30%	.
„ 10	*580*	– 6%	–	.
„ 10	*524*	– 6%	–	.
„ 10	*578*	tr 15%	–	.
„ 10	*522*	+ + +	+ 30%	.
„ 11	*578, 522, 524*	–	–	.
„ 21	597, *578, 580,* 442	–	.	–
„ 21	*515*	+ + + ±		+ + + ±
„ 21	557	–	.	+ + + +
„ 22	*578, 580, 522, 524*	–	.	–
„ 22	557	–	–	+ + + +
„ 22	*515*	+ 30%	+ 40%	+ + + ±
„ 22	516	+ 30%	– 6%	–
„ 22	544	+ + + ±	+ 35%	–
„ 26	*515*	–	+ 20%	+ 33%
„ 26	557	.	–	+ + + +
„ 26	Okero, Dom, G	.	–	–
„ 27	*515*	tr 12%	+ 25%	+ + +
„ 27	557	–	–	+ + + +
„ 27	612	–	–	+ 40%
„ 27	597	–	–	–
„ 27	614	–	–	+ + +
„ 27	*578*	–	–	+ + +
Dec. 9	Okero, Dom, E, G, 516	–	–	
„ 9	544	+ + + +	–	

Remarks on the T. rhodesiense *tables.*

From these *T. rhodesiense* tables we learn:

(1) That a negative reaction justifies no conclusions; the same two bloods tested again a few days later may give a strongly positive reaction.

(2) That the blood of an infected animal shortly after the administration of Bayer 205 reacts freely with *T. rhodesiense* from either recent or more chronic infections. After the lapse of several weeks the blood becomes less sensitive until its response is only called forth, and then often feebly, by trypanosomes from very early infections.

(3) That the same strain of trypanosome in consecutive passages in different individuals of the same species of animal behaves differently when tested with one and the same blood. Evidently we have no strict parallel to the "passage" and "relapse" strains of workers with European laboratory strains. This is shown by the fact that a passage strain often evokes a response in a blood whose history makes it perfectly clear that it is a "relapse" blood, *sensu strictu.*

(4) That of the six adhesin bloods shown in Table VII, only one, M 515, reacted with *T. gambiense* trypanosomes. The blood of this monkey reacted on occasions strongly both with *T. gambiense* and with the Damba strain. This monkey 515 was also the only *T. rhodesiense* animal to react with the trypanosomes of strain Okero, and then only to 50 per cent. Evidently the first five monkeys gradually lost the adhesins evoked by the administration of Bayer, whereas 515 being superinfected and again treated acquired a new lease of activity.

(5) M 515 was reinfected by flies carrying a different passage of its original infecting strain of *T. rhodesiense.* At the time of its reinfection its blood showed strong reactions with *T. rhodesiense,* thus showing that the presence of adhesins are not necessarily associated with immunity against reinfection with the same strain of trypanosome.

(6) That the heavier infections caused by *T. rhodesiense* in both monkeys and guinea-pigs are not accompanied by more strongly marked or more easily evoked reactions. On the contrary, the response of this trypanosome both in monkeys and in guinea-pigs is less regular than that of *T. gambiense.*

(7) That in the guinea-pig the reaction is very much more irregular than in the monkey.

(8) The trypanosomes of guinea-pigs 6 q and 6 r behaved differently. 6 r never reacted with any adhesin blood whereas 6 q did, though not strongly. Monkeys 620 and 621 were inoculated from 6 q and 6 r respectively; the trypanosomes of 621 responded to adhesins much more readily than those of 620.

Section 3. Animals infected with the Damba strain.

Table IX.

Genealogy.

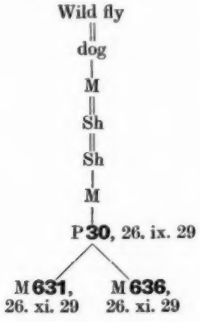

Table X.

Three passages of the Damba trypanosome tested with different positive adhesin bloods.

Date	Adhesin blood	Tryp. blood 631	636	P 30
Dec. 2	*515, 578, 580, 524,* 544	·	·	–
„ 4	*515*	tr 15%	·	·
„ 4	*578*, 513, 516	–	·	·
„ 4	544	+ 26%	·	·
„ 6	601, 612, 544, 610	·	·	–
„ 7	612	tr 17 %	·	·
„ 7	544, 597, *515*, 516, 513, 557, *578*, *580*	–	·	·
„ 10	612	+ + + +	·	·
„ 10	544	–	·	·
„ 10	624	–	·	·
„ 11	513, 544	+ + + ±	·	·
„ 11	612	+ 38%	·	·
„ 11	516	+ + + +		·
„ 11	*515*	+ +		
„ 11	*522, 578, 580, 585,* 597, 557, 624, 381, 451	–	·	
„ 13	Okero, Dom, G, E, 456, 256, 410	–	·	
„ 16	612	tr 18%	+ 40%	–
„ 16	513	– 8%	+ + + +	–
„ 16	544	+ 25%	+ + + ±	+ +
„ 16	*515*	–	–	+ 30%
„ 16	516	+ 25%	–	+ 45%
„ 20	451	·	·	+ 25%
„ 20	513, 516, 544, 488, 487, 381	·	·	–
„ 24	*515*	·	+ +	·
„ 24	516, 633	·	–	·
„ 24	544	·	+ 25%	·
„ 24	513	·	tr 13%	·

Section 4. T. congolense.

The single *T. congolense* strain used in these experiments was obtained by exposing a dog to wild *G. morsitans* in the West Nile area.

This trypanosome shows a tendency in fresh coverslip preparations of blood to adhere to the corpuscles, a phenomenon especially noticeable in slides prepared from infected dogs. Dilution of the blood with citrate to a great extent eliminates this feature, but there is no doubt that in our employment of the adhesion test especial care must be devoted to controls. It is not necessary to give the *T. congolense* tests in detail. Some of the controls showed red-cell adhesion up to 8 per cent., and in each of these instances in addition there were a few trypanosomes with single platelets sticking to them. Apparently this species is definitely more prone to stick to objects in the blood, and in this respect it is reminiscent of the behaviour of *T. uniforme* in fresh blood preparations.

The strain was tested on several occasions against those monkeys whose blood showed best the tendency to respond with more than one "species" of trypanosome, *i.e.* 513, 515, 516, 544, 612 and 624. The highest response was 8 per cent. red-cell adhesion and 3 per cent. platelet adhesion given once by the blood of a dog against 516. Tested against a wild pig that had been for four months infected with *T. gambiense* the response on three different occasions was 80 per cent., 50 per cent. and 8 per cent. This pig had been sent to the Laboratory by the Game Warden and was captured near Jinja in the Eastern Province. It is probable that it had previously been infected with *T. congolense* as this trypanosome is common throughout the Protectorate.

The main importance of this section is to show that:

(1) *T. congolense* did not call forth a response with these very sensitive monkey bloods.

(2) That a wild pig's blood gave a very strong response suggesting that the animal had suffered from an infection with this trypanosome.

Part II.

Section 1. *Selected human cases studied at the Laboratory Hospital.*

Musa. A Nilotic native found in the neighbourhood of Jinja; impossible to say whether he was infected in Northern Province (West Nile area) or Eastern Province (Lake Victoria—Mpologoma area). (Cured.)

Blasio. A laboratory attendant who developed the disease in 1927 at Entebbe. Presumably infected in the course of his work by being accidentally bitten through the wire of a box of infected flies. (Cured.)

Okero. From near Mutir in the West Nile area. Sent to Entebbe as an arsenic-resistant case. (Under treatment.)

Dominiko. Same as Okero; had also resisted treatment with tryponarsyl.

Eli. From Madi, Gulu district near the Nile. Has resisted a "full course" of tryparsamide.

Girisomu. (G.) A native of Busoga found infected near Jinja. (Under treatment with tryparsamide; great improvement.)

Of these strains Okero, Dominiko (Dom), and Eli (E) were subinoculated into clean monkeys.

The genealogies of strains Okero and Dominiko are as follows:

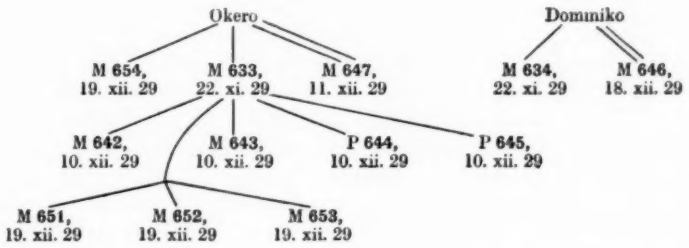

Table XI.

Reactions of the bloods of these patients with various tryp. bloods.

(Sections 1 and 2.)

Section 1.

S.S. Patient, Musa			Blasio		
Date	Tryp. blood	Adhesion	Date	Tryp. blood	Adhesion
Oct. 21	P 17	+ +	Oct. 15	P 17	+ 20%
„ 21	P 25	+	„ 15	P 25	+ +
„ 21	*609*	+ +	„ 20	P 17 d	−
„ 21	P 17 d	+	„ 20	P 17 e	− 10%
„ 22	P *6* r	− 7%	„ 22	*609*	−
„ 22	*609*	+ + + +	„ 22	P *6* q	−
„ 22	P *6* q	−	„ 22	P *6* r	−
„ 23	*609*	−	„ 28	P 17 d	−
„ 23	P 17 d	tr 15%	„ 28	P 17 e	− 10%
„ 23	P 25	−	„ 28	P *34*	−
„ 24	P 25	tr 10%	Nov. 8	623	+ 40%
„ 24	P 17	+ + + ±	„ 18	*619*	−
„ 24	P 34	+	„ 29	624	+ + +
„ 27	613	tr			
„ 27	P 17 e	+ 46%			
Nov. 6	P 17 e	+ 46%			
„ 6	P 17 d	+ 30 %			
„ 6	P 25	+ 30%			
„ 8	606	+ +			
„ 9	*617*	+			

Section 2.

Date	Tryp. blood	Okero	Dominiko	Eli	Girisomu
Nov. 11	P 25	−	−	.	.
„ 11	P 17 e	+ + +	−	.	.
„ 11	P 17 d	+ +	−	.	.
„ 11	606	+ + + +	+ + + +	.	.
„ 11	623	+ + +	+ + + ±	.	.
„ 12	606	+ 25%	tr 13%	.	.
„ 14	*619*	−	+ 30%	.	.
„ 15	P 17 g	+ 45%	+ +	.	.

Table XI (*cont.*).

Section 2 (*cont.*).

Date	Tryp. blood	Okero	Dominiko	Eli	Girisomu
Nov. 15	P 17 da	+ + + ±	–	.	.
„ 18	*619*	+ 46%	tr 10%	.	–
„ 19	*621*	– 9%	–	.	–
„ 19	*619*	+ + +	+ + +	.	–
„ 19	P 25	–	–		–
„ 26	*620, 621*	–	–	.	–
„ 26	P 17 g	+ + + ±	+ 40%	.	–
„ 29	624	+ + + +	+ +	.	+ +
„ 29	P 25	–	–	.	–
Dec. 3	**633**	+ + +	–	.	–
„ 4	**633**	+ + + ±	–	.	–
„ 4	614	+ 27%	–	.	–
„ 5	**633**	+ + + ±	–	.	–
„ 6	**633**	+ +	–	.	–
„ 6	P 25	+ + +	–	.	–
„ 9	P 17 g	–	+ 26%	–	–
„ 9	614	+ + + ±	+ + + +	–	–
„ 9	**633**	+ + + ±	–	–	–
„ 13	**631**	–	–	–	–
„ 13	**633**	+ + +	–	–	–
„ 13	P 17 g, P 25, P 17 h	–	–	–	–
„ 13	P 17 j	+ + +	.	.	.
„ 18	**633**	+ + +	+ + + +	+ 45%	+ 40%
„ 19	P 25	–	–	–	–
„ 19	P 17 g, P 17 h	–	+ +	–	–
„ 19	**642, 643**	+ + +	–	–	–
„ 20	632	–	–	+	– 25%
„ 24	628	+ + +	+ + +	+ +	–
„ 24	*638*	–	–	–	–
„ 29	**647**	+ + + ±	–	–	–
„ 30	**654**	– 9%	–	–	–
„ 30	**653**	+ + +	+ 25%	–	–
Jan. 2	646	tr 10%	+ +	–	
„ 2	**647**	–	–	–	–
„ 8	646	+ +	+ + +	+ 25%	–

Table XII.

Trypanosomes of strain Okero tested with various adhesin bloods.

Date	**633** tryp. (strain Okero). Adhesin blood	Adhesion
Dec. 3	507, 544, 601, 610, 451, Dom, G	–
„ 3	Okero	+ + +
„ 4	513, 557, Dom, G, *580, 585, 578,* 507, 544	–
„ 4	Okero	+ + + ±
„ 4	516	– 6%
„ 4	*515*	+ 26%
„ 5	516, Dom, G, E, 544	–
„ 5	Okero	+ + + ±
„ 5	513	– 9%
„ 5	*515*	+ +
„ 6	544, 507, Dom, G, E, 513, 516	–
„ 6	427	– 8%
„ 6	Okero	+ +
„ 7	544, 612, 597, 516, 513, *578, 580*	–
„ 7	*515*	+ 25%
„ 9	Okero	+ + + ±
„ 9	Dom, G, E	–
„ 10	Okero	+ + +
„ 10	Dom	+ + + +
„ 10	G	+ 40%
„ 10	E	+ 45%

Remarks on Tables XI and XII.

A feature of Okero's and to a less extent of Dominiko's trypanosomes is the regularity of their reaction with the blood of the patient from which they were isolated. This has been confirmed by observations with a number of experimental animals.

The diagnosis of Okero's strain will be the subject of a separate report. It may be remarked here that this strain is indistinguishable by laboratory tests from *T. rhodesiense*, being indeed the first example of this form of trypanosomiasis known to have been contracted within the limits of the Uganda Protectorate. Strain Dominiko in experimental animals behaves like *T. gambiense*, so do strains Eli and Girisomu. These last two are remarkable for their general failure to react, save on rare occasions and then only partially.

The adhesion reactions of Dominiko's blood are shown in Table XI: his trypanosomes, as shown in Table III, reacted to 100 per cent. with the bloods of monkeys 513, 516 and 544 to 90 per cent. with monkey 487 and to 40 per cent. with monkey 612.

Section 2. An examination of human cases of trypanosomiasis in the West Nile district of Uganda, using the red-cell adhesion test.

In addition to the above detailed study of the patients in the Laboratory Hospital we decided to test a number of cases at least equal to that examined by Johnson and Lester, who used the adhesion test as originally described.

The only area in the Protectorate where this number of cases could be obtained was the West Nile, and one of us (J. M. W.) accordingly visited this area, including in his travels the Aringa district where the disease has recently been spreading. Two hundred and sixteen cases were tested and in nearly all of them the same infected animals were used as the source of trypanosomes.

Eight guinea-pigs were taken, four infected with *T. gambiense* (Nos. 17 d, 17 e, 25 a and 25 b) and four with *T. rhodesiense* (Nos. *33* a, *33* b, *6* s and *6* t). Monkey 623, infected with *T. gambiense*, was also taken, but during the tour its blood never showed trypanosomes in sufficient numbers for the test.

The patients were divided into three groups: I, untreated, II, during treatment and III at least two months after treatment commenced. (Only two cases, one European and one native, were available more than a year after treatment, and they gave completely negative tests with *T. gambiense* and *T. rhodesiense*.)

Table XIII gives a summary of the results.

All these cases were diagnosed by gland puncture, performed by specially trained natives.

Among the five untreated cases in Group I that gave no adhesion reaction were three in whose gland juice trypanosomes were seen by one of us (J. M. W.) at the time the test was made.

Table XIII.

	100% ++++		99–90% +++±		89–60% +++		59–50% ++		49–20% +		19–10% Tr.		9–1% Neg.		0 0		Total Cases
Group I.		%		%		%		%		%		%		%		%	
Untreated G	*1*	5·2	*5*	26	*6*	31·2	*2*	10·4	—		—		—		*5*	26	*19*
R	—		—		*3*	17·6	—		*2*	11·7	—		*2*	11·7	*10*	59	*17*
Group II.																	
During treatment G	*10*	5·6	*23*	12·9	*26*	14·6	*6*	3·3	*11*	6·2	*2*	1·1	*6*	3·3	*93*	52·5	*177*
R	—		—		*2*	2·6	*1*	1·3	*5*	6·6	*2*	2·6	*1*	1·3	*64*	85·3	*75*
Group III.																	
At least two months *after* treatment G	—		*1*	5	*4*	20	—		*2*	10	*2*	10	*2*	10	*9*	45	*20*

Total positive reactions (*i.e.* 50% adhesion and over): *T. gambiense* 84/216 38·8%
" " " " *T. rhodesiense* 6/92 6·5%

In addition to the above, eleven untreated cases were diagnosed as suspicious by gland palpation, but showed no trypanosomes in the gland juice and gave negative adhesion tests.

These eleven cases are not included in the table, nor are a further twenty-three treated cases whose blood was not examined on the day it was collected, as was usual, but was kept till next day.

Thirteen of these latter gave negative reactions, two less than 10 per cent., two a trace, four +, two +++; that is, two out of twenty-three were definitely positive.

Comparing these with the last twenty-three bloods examined on the previous day, we get: nine negatives, three +, one ++, six +++ and four +++±, or eleven good positives out of twenty-three.

The first series of bloods was kept at room temperature in glass tubes with plasticine plugs, no special precautions being taken to keep the blood sterile when it was collected.

One blood was taken on 20. xi. 29 and gave a +++ adhesion with P 17 e. It was collected carefully into a sterile corked tube. This blood, kept till next day, again gave a +++ reaction. It remained untouched till 27. xi. 29, when it was again tested and still gave slight adhesion with P 17 e trypanosomes, though by this time it was discoloured and foul-smelling.

Our system of recording results has been already explained, but it may be mentioned here that a complete negative means that at least twenty consecutive trypanosomes seen were free from cells; if one was seen adhering at least a hundred were counted.

It is not necessary to give a detailed list of the individual reactions, but the following points are of interest:

1. Of patients tested with *both* trypanosomes none reacted to *T. rhodesiense* that did not also react to *T. gambiense.*

2. A reaction to *T. rhodesiense* was given:

by 7 out of 12 cases, 58%, in Group I;
by 11 out of 75 cases, 14%, in Group II.

Group III was not tested with *T. rhodesiense.*

3. Reacting to *T. gambiense* only, though tested with both trypanosomes, were:

5 out of 12, 41·6%, in Group I;
64 out of 75, 85·3%, in Group II.

4. Only three cases gave slightly higher figures with *T. rhodesiense* than with *T. gambiense*, viz.:

1 in Group I gave	72%	adhesion with *T. rhodesiense* and	54%	with *T. gambiense*.
2 others in Group II gave	68%	" " "	65%	"
	and 28%	" " "	24%	"

Controls.

As is shown in Part I, the degree of reaction between a given tryp blood and adhesin blood may vary greatly from day to day, so that in every diagnostic test the sensitiveness of the trypanosomes should be controlled. In employing the test for diagnosis the ideal procedure would be to test the tryp blood against a known positive adhesin blood every day that an unknown blood is under examination. Unfortunately this was not possible in the field. On the other hand, controls against non-specific adhesion were employed freely as follows:

(*a*) *In the field.* Five hospital patients, believed to be free from trypanosomiasis, were selected. Four were suffering from chronic ulcers, probably syphilitic, and were receiving neotrypanarsyl. The fifth was an undiagnosed and untreated febrile case. All were tested against the trypanosomes of guinea-pig 17 d: two gave completely negative reactions and two 2 per cent. and 6 per cent. respectively. The febrile case was completely negative.

(*b*) *At Entebbe.* Here further controls were carried out on one hundred individuals whose history and circumstances justified the designation "normal." They comprised forty-nine native prisoners, forty-two native laboratory employees and nine Europeans. All were apparently healthy, though undoubtedly many of the natives had at one time or other suffered from both syphilis and malaria.

With *T. gambiense* 4 per cent. of these gave non-specific reactions varying from 2 to 4 per cent.; 8 per cent. gave reactions with *T. rhodesiense* of from 1 to 4 per cent.

They were all tested against trypanosomes from the same guinea-pigs, 17 g and *33* d. Control tests on the sensitiveness of the trypanosomes in these two animals gave the following results from day to day during the performance of these controls on normal men:

17 g tested with M 544: ++++; ++++±; ++++±; +++; ++++±; ++++±; ++++±; ++++±.
33 d tested with M *638*: +++; +++; +++; ++; ++; ++; +.
33 d tested the first day with M *515*: ++++.

A noteworthy fact about the results of these tests with human beings is that a positive reaction was never given by anyone whose history precluded

exposure to tsetse. On the other hand, cases of undoubted trypanosomiasis gave a negative reaction.

Bearing in mind the variability exhibited by the experimental animals in Part I, it is rather remarkable that so high a percentage of positive results was obtained when only one examination was made of each case. In field work involving large numbers of cases this is indeed a grave difficulty, and here we are once again impressed with the danger of reliance on a single negative test.

Another practical difficulty is that the infected animal may suddenly enter a negative phase and so cut off the supply of trypanosomes.

A rough control of the sensitiveness of the trypanosome employed was afforded, in the case of *T. gambiense*, by the fact that on no day when the blood of guinea-pig 17 e was employed did we fail to get at least one positive reaction.

An attempt was made to ascertain if there was any correlation between the percentage of positives and the distribution of cases throughout the district.

Of one hundred and eighty cases examined in one place, forty-four lived under the same headman and therefore presumably resided within a few miles of one another. Twenty of these forty-four (47·7 per cent.) gave positive adhesion tests with *T. gambiense* (50 per cent. adhesion or over).

Three other groups of forty-four, selected at random and living in other districts often as far as twenty miles apart were also examined. These three groups gave respectively twelve, fifteen and fourteen positive adhesion reactions, *i.e.* an average per group of 31 per cent. These figures do not convey much information, and it would indeed be surprising if there were any strict localisation of trypanosome strains, as the population is in constant movement and communications are easy.

The only way to compare satisfactorily the red-cell adhesion test with the test as carried out by Johnson and Lester would be to make a duplicate series of tests on the same bloods. Unfortunately this was not possible in the time at our disposal.

For work in the field the red-cell adhesion test has several advantages over its predecessors: it requires less apparatus and takes less time. Of the relative accuracy of the two methods we cannot yet speak with authority.

It is instructive to compare, as far as is possible, our figures obtained with human cases with those of Johnson and Lester:

	Our figures	Sherifuri figures
Group I	73·6% of 19 cases	90% of 30 cases
„ II	31% of 177 cases	86·5% of 37 cases
„ III	25% of 20 cases	84·6% of 52 cases

There are, however, several reasons why these figures are not directly comparable.

1. Johnson and Lester did not test their *T. gambiense* strain against known infected animal bloods before doing their series of tests with human blood.

2. Our trypanosomes were taken from a guinea-pig for the purposes of the test: Johnson and Lester's were presumably taken from a monkey, though they do not say so definitely in their paper.

3. Their strain of *T. gambiense* seems quite different from our strains. They do not give the history of their strain, the number of animal passages it had undergone or whether syringe transmission or fly transmission was used.

They state, however, on page 1 of their paper, that "For preparation of the trypanosome suspension it is convenient to use a blood showing trypanosomes from 20–30 per field under a 1/6th objective." This means probably 10–15 trypanosomes per field as examined when mixed with the test plasma.

Only very rarely have we met with infections as numerous as this, even in guinea-pigs and certainly never in monkeys, so there is obviously a very great difference in the virulence of the strains used.

4. The system of recording the results is different. For reasons given in the Introduction, Section (*g*), we do not use Brussin's method.

For diagnostic purposes we regard as positive reactions ++ and upward, that is at least 50 per cent. of the trypanosomes showing adhesion. Brussin's ++−−− is very vague, but his +++−− is described as meaning that half the total number of trypanosomes is adherent and so corresponds to our ++.

If we include in the Sherifuri "positives" only those showing the same degree of adhesion as our "positive" cases their figures appear thus:

Group I 66·6%. Group II 83·7%. Group III 69·2%.

With this alteration the figures for Group I come fairly near to our Group I; but there is still a great difference between the figures in Group II—the cases during treatment. We cannot explain this.

It is interesting to note that none of our untreated cases gave doubtful reactions. They all gave either more than 50 per cent. adhesion or none at all.

With six of our West Nile patients the test was repeated a second time. Two of these were treated cases giving a +++ reaction when first tested: when re-examined the next day the reactions were respectively ++++ and +++.

The other four, all untreated and all with trypanosomes demonstrable in their gland juice, gave negative reactions when first tested. One was retested on the next day and was again negative. The following day she received an injection of trypanarsyl and twelve days later was tested for the third time and again found negative. Two others, having each received an injection of trypanarsyl the day after the first test, gave a negative reaction six days later.

The fourth case, also treated the next day, gave twelve days later another negative test.

Summary of Section 2.

1. Two hundred and sixteen cases of human trypanosomiasis were tested for the red-cell adhesion test with *T. gambiense*. These were divided into Group I (nineteen untreated cases), Group II (one hundred and seventy-seven during treatment) and Group III (twenty at least two months after treatment).

The positive results were 38·8 per cent. of the total: 73 per cent. of Group I, 31 per cent. of Group II, 25 per cent. of Group III.

2. Seventeen in Group I and seventy-five in Group II were also tested with *T. rhodesiense*. The positive results were 6·5 per cent. of the totals: 17·6 per cent. of Group I and 3·9 per cent. of Group II.

3. More than one hundred control tests were made with normal natives and Europeans.

4. The red-cell adhesion test and the ordinary adhesion test are compared as far as the results available are comparable.

5. An interesting point is the occurrence of positive reactions to *T. rhodesiense* in 6·5 per cent. of ninety-two cases, treated and untreated. Possible explanations of this will be discussed at the end of the paper.

6. Reasons are given why the results of Lester and Johnson are not really comparable with the figures given for the red-cell adhesion tests in this paper.

Part III. An analysis of the mechanism of the adhesion test.

While testing the blood of various animals by the red-cell adhesion test (see Part I) it was noticed that the blood of guinea-pigs gave strong platelet adhesion but *not* red-cell adhesion.

This fact, coupled with the observation that agglutination of red-cells tended to occur when the blood of two different animal species was used, focussed our attention on the red-cell element in the reaction and led to experiments to find out more about its mechanism.

The components of the reaction are as follows:

Adhesin blood	red cells (A.R.C.).
	plasma (A.P.).
Tryp. blood	red cells (T.R.C.).
	plasma (T.P.).
	trypanosomes (T.).

These components can be separated[1] and recombined to study the part played by each one.

Thus the red cells in either adhesin blood or tryp. blood (A.R.C. or T.R.C.) can be removed by centrifugalisation of the citrated blood leaving the plasma (A.P. or T.P.).

The trypanosomes (T.) cannot be obtained in the same degree of purity as the other components, for the action of the centrifuge and washing causes a

[1] Platelets and blood dust of course occur in both adhesin and tryp. blood. The methods we employ do not lead to their removal, so that they are found in each of the five components.

A few red cells are found in the plasma (A.P. and T.P.) and also in the trypanosome suspension but never in sufficient quantities to cause confusion.

As mentioned in Part I, when looking at "caddis forms," we cannot always be certain that red cells are not adhering to trypanosomes by means of platelets, but when single red cells adhere it can readily be seen that there is nothing intervening.

heavy mortality among them; but they can be obtained almost entirely free from red cells (T.R.C.) by *slow* centrifugalisation.

Tryp. plasma (T.P.) can be obtained free from trypanosomes (T.) and red cells (T.R.C.) by rapid spinning; tryp. blood can be freed from trypanosomes by heating to 56° C. for thirty minutes. Inactivation by heat can be used to separate the elements of the reaction and also to see what effect it has *per se* on any of them.

Of the five components (A.R.C., A.P., T.R.C., T.P., and T.) two only, A.R.C. or T.R.C., can be left out without interfering with the reaction.

If we calculate the number of possible experiments in which we remove one or other of these two components, we find that there are twelve possible arrangements using guinea-pig and monkey as the source of tryp. blood and adhesin blood.

In addition to these "removal" experiments there are four more in which T.R.C. can be *replaced* by normal red cells of the same species.

As well as these sixteen combinations there are two others in which a normal monkey's cells are substituted for guinea-pig T.R.C. in a system of guinea-pig components, and, conversely, guinea-pig cells in a monkey system. We can also use the cells of normal individuals of other animal species in this way.

It will readily be seen that the complete investigation of the mechanism of the red-cell adhesion reaction on these lines involves much study, and we have only been able to try nine out of these eighteen possible combinations. (See Table XIV.)

It must be remembered in explanation of these omissions that we only very occasionally find sufficient trypanosomes present in a monkey infected with our strains of *T. gambiense* to enable us to do more than the simple red-cell adhesion test using citrated whole blood.

In monkeys infected with *T. rhodesiense* there are, as a rule, sufficient trypanosomes, but the animals do not live long untreated.

Unfortunately too the guinea-pig is not a suitable animal for the production of an adhesin plasma. Of those tested some had received doses of Bayer or atoxyl in the course of other experiments, and others were treated with Bayer *ad hoc*.

The only two guinea-pigs to give more than 50 per cent. adhesion were 16 a and 17 c (see Part I).

The above considerations explain why most of our experiments have been done with adhesin blood from monkeys and tryp. blood from guinea-pigs.

In another experiment, not shown in Table XIV, but using a similar system of components to No. 4, we removed the guinea-pig cells (T.R.C.) and replaced them by washed red cells from the following normal animals: European; *Cercopithecus* monkey; baboon; sheep; goat; calf; wild-pig; domestic cat; rabbit; rat and guinea-pig.

The red cells of the three Primates adhered, giving typical "caddis forms";

Table XIV.

Variations of the adhesion test obtained by omitting certain components and substituting others.

Experiment No.	Components					Result
	A.P.	A.R.C.	T.P.	T.	T.R.C.	
1.	M	M	P	P	P	The usual test; see Part I for examples
2.	M	M	P	P	Removed	Red-cell adhesion
3.	M	Removed	P	P	P	Red-cell adhesion of guinea-pig cells
4.	M	Removed	P	P	Removed and replaced by normal washed P cells	No adhesion
5.	M	M	M	M	M	Red-cell adhesion; the usual test; see Part I for examples
6.	P	P	M	M	M	Red-cell adhesion
7.	P	P	P	P	P	Platelet adhesion only
8.	P	Removed	P	P	Removed and replaced by normal washed P cells	Platelet adhesion only
9.	P	Removed	P	P	Removed and replaced by normal M cells	Red-cell adhesion

Note. In this table M (monkey) and P (guinea-pig) indicate the animals from which the components were derived.

on the other hand, none of the others gave red-cell adhesion, except an occasional single cell adhering (see Experiment 10).

This observation, together with its apparent contradiction (see Experiment 3) in which guinea-pig cells *did* adhere, seems to us so important that we include it in this paper without waiting for the completion of the series of possible experiments.

As indicated earlier, the performance of some of these combinations involves more than the mere planning of the experiment; it means waiting for the lucky chance of a monkey having a sufficiently heavy infection with *T. gambiense* to enable us to make a trypanosome suspension free from red cells.

In addition to the above analytical experiments it was thought desirable to compare the results of the red-cell adhesion test with the ordinary adhesion test, using the same blood in each.

As already stated, it was not convenient to make the duplicate test in the field, using human bloods, so we were limited to monkeys with chronic infections and to one human case, Okero.

Using M 544 and trypanosomes from P 25a, with Johnson and Lester's technique, no adhesion whatever was seen; with our technique $+++\pm$ red-cell adhesion was obtained.

Okero's blood did not react with P 25a trypanosomes but it did so with those from M **651** infected with Okero's own strain.

It gave a positive result with both methods but a stronger one with the red-cell adhesion test (see Experiment 12).

Finally we found that adhesin blood after inactivation is only slightly less powerful in causing red-cell adhesion, thus confirming the work of other investigators who found that platelet adhesion was similarly unaffected (see Experiment 13).

There now follows a fuller account of the actual experiments given in Table XIV.

Experiment No. 1. See Part I for examples.

Experiment No. 2. 5. xi. 29. *The test after removal of* T.R.C.

Equal parts of citrated whole blood of M 544 and suspension of P 17 d trypanosomes.

Result: ++++ red-cell adhesion, all "caddis forms."

Experiment No. 3. 5. xi. 29. *The test with* A.R.C. *removed.*

Equal parts of citrated whole blood of M 544 *centrifuged clear of cells* and citrated whole blood of P 17 d.

Result: ++++ red-cell adhesion, all "caddis forms."

Experiment No. 4. 5. xi. 29. *The test with* T.R.C. *removed and replaced by normal guinea-pig red cells* (A.P. *from monkey*).

Equal parts of citrated whole blood of M 544, *centrifuged clear of cells*, normal guinea-pig red cells washed twice in citrate saline, and 17 d tryp. suspension.

Result: No red-cell adhesion; scanty platelet adhesion and glass adhesion.

Experiment No. 5. See Part I for examples.

Experiment No. 6. 6. i. 30. *The test using adhesin blood from guinea-pig and tryp. blood from monkey.*

Equal parts of citrated whole blood from P 16 a, and citrated whole blood from M 628.

Result: +++ red-cell adhesion.

Experiment No. 7. 28. x. 29. *The test with guinea-pig components only.*

Equal parts of citrated whole blood of P 16 a, and citrated whole blood of P 17 e.

Result: ++++ platelet adhesion.

(Some adhesion of red cell by means of platelets, some leucocytes adherent with or without platelets, many tryps adherent to glass by means of platelets but *no red cells adherent direct.*)

Experiments Nos. 8 and 9 show the difference between guinea-pig cells and monkey cells when acted on by guinea-pig adhesin plasma.

Experiment No. 8. 1. xi. 29. *The test with* T.R.C. *removed and replaced by red cells of a normal guinea-pig.* (A.P. *from a guinea-pig.*)

Equal parts of citrated whole blood of P 16 a, *centrifuged clear of cells*, citrated blood of normal guinea-pig, and trypanosome suspension from P 17 d.

Result: +++± platelet and leucocyte adhesion only.

Experiment No. 9. 1. xi. 29. *The test with* T.R.C. *removed and replaced by normal monkey red cells.* (*All other components from guinea-pigs.*)

Equal parts of citrated whole blood of P 16 a, *centrifuged clear of cells*, citrated blood of normal monkey, and trypanosome suspension from P 17 d.

Result: ++++ red-cell adhesion.

Experiment No. 10. 4. xi. 29. *The test using the red cells from individuals of various animal species.*

"544 plasma" means citrated blood of M 544 centrifuged clear of cells.

"17 d tryp." means citrated blood of P 17 d centrifuged *slowly* and the suspension of trypanosomes pipetted off.

Control: Equal parts of M 544 citrated whole blood and P 17 d citrated whole blood.

Result: ++++ red-cell adhesion—"caddis forms."

(The trypanosome suspension was seen to contain a few red cells and platelets. A few cells were also seen in 544 plasma.)

Red cells of *Cercopithecus* monkey, baboon, bush-pig, domestic cat, white rat, and guinea-pig were examined after twice washing in citrate saline and centrifuging. Platelets and leucocytes were seen in the suspension of red cells of baboon, bush-pig and domestic cat; leucocytes and no platelets seen among red-cells of guinea-pig, rat and monkey.

Tests	Results
Equal parts of 544 plasma, 17 d tryp., and rat cells	No "caddis forms," a few single red cells adherent,
monkey cells	++++ red-cell adhesion,
baboon cells	++++ red-cell adhesion,
cells of bush-pig "Horace"	No "caddis forms"; a few single red cells only,
cells of cat "Felix"	No "caddis forms"; a few single red cells only,
cells of guinea-pig	No "caddis forms"; no red cells adherent.

Experiment No. 11. 10. i. 30. *Tests with monkey and human bloods, using the technique of* Johnson and Lester.

Control 1: Equal parts of citrated whole blood of M 544 and citrated whole blood of P 25 a.

Result: ++++± red-cell adhesion.

Control 2: Okero's citrated whole blood and P 25 a citrated whole blood.

Result: Non-specific adhesion only (2 per cent. single red cell only).

Control 3: Equal parts of normal human (J. M. W.) citrated whole blood and P 25 a citrated whole blood.

Result: No adhesion.

Test: Citrated bloods of M 544, normal human (J. M. W.) and Okero centrifuged hard and the clear plasma pipetted off.

Citrated blood of P 25 a centrifuged *slowly* and trypanosome suspension pipetted off.

Two drops of each of the three plasmas mixed with two drops of trypanosome suspension and incubated for 40 minutes at 37° C. in small glasss tubes covered with plasticine to prevent evaporation. Samples were then examined microscopically.

Result: No adhesion of any kind in M 544, Okero or J. M. W. (Numerous platelets and a few red cells seen quite free from trypanosomes.)

Experiment No. 12. 11. i. 30. *Further adhesion tests with Okero's blood.*

Control: Equal parts of Okero's citrated whole blood and citrated whole blood from **M 651**.

Result: +++ red-cell adhesion; no "caddis forms."

Test: Okero's citrated blood centrifuged clear, plasma pipetted off and two drops mixed with two drops of M 651 tryp.-suspension made by slow centrifuging of citrated blood. The mixture incubated for thirty minutes at 37° C. as before. A sample then examined.

Result: Platelet adhesion to 30 per cent. of trypanosomes.

Controls after test: Okero plasma with M 651 citrated blood gave +++± red-cell adhesion including many "caddis forms." Okero's plasma with washed normal human red cells and **M 651** trypanosomes gave +++ red-cell adhesion. Okero's plasma with washed normal guinea-pig red cells gave no adhesion.

Experiment No. 13. 31. x. 29. *Effect of inactivation on adhesion* (*T. gambiense*).

Control: Equal parts of citrated whole blood of M 544 and citrated whole blood of P 17 d.

Result: ++++ red-cell adhesion.

(Repeated with same result after M 544's blood had been left on the bench for fifty minutes.)

Test: M 544 blood heated in a water bath for forty minutes at 53° to 55° C. and mixed with equal parts of citrated whole blood of P 17 d.

Result: ++++ red-cell adhesion.

Continued on 1. xi. 29 (*T. rhodesiense*).

Control (before inactivation): equal parts of M *580* plasma and P *23* a trypanosome suspension.

Result: + red-cell adhesion (21 per cent.).

Test: Equal parts of M *580* plasma (inactivated as above) and P *23* a trypanosome suspension.

Result: + red cell adhesion (20 per cent).

Discussion.

The first point to be considered is the relationship between red-cell adhesion and platelet adhesion. Are the two phenomena different manifestations of the same substance in the plasma?

In favour of this are the following:

1. The fact that adhesion of something to trypanosomes occurs in both.

2. The substance is heat stable; inactivation of the blood or plasma will not prevent either reaction.

3. The same blood gives positive results in both tests (see Experiment No. 11).

4. In both series of human cases, Johnson and Lester's and our own, there are instances of undoubted trypanosomiasis failing to give a positive reaction.

5. Using the same guinea-pig plasma (16 a) and suitable trypanosomes, we can get either red-cell adhesion or platelet adhesion in different circumstances (see Experiments Nos. 8 and 9).

6. Some monkey bloods, *e.g.* 516 with P 17 d and P 17 e tryp. bloods, gave reactions in which it was clearly seen that platelets and red cells were adherent to the same trypanosomes (see Table IV).

Against the two reactions being due to the same substance are:

1. The failure of plasma of M 544 to cause adhesion of staphylococci or yeasts to trypanosomes (see Experiment No. 10).

2. The failure of M 544 plasma to give platelet adhesion when Johnson and Lester's technique was used, though it gave a $+++\pm$ red-cell adhesion with our technique.

We believe that the two reactions are due to the same hypothetical substance, "adhesin," in the plasma, but until more work is done on the factors determining the adhesion of guinea-pig cells we cannot say if there is more than one substance concerned.

There appears to be something in monkey plasma that enables guinea-pig cells to adhere, for without this element, which is probably also present in other primates, they seem unable to do so.

Experiment No. 11 suggests that red-cell adhesion is more sensitive than platelet adhesion.

The experiments detailed above partly explain why other observers did not emphasise red-cell adhesion.

Johnson and Lester, although they employed monkeys as a source of trypanosomes in their tests with human blood, centrifuged the blood free from cells. Leupold, and Brown and Davis, used rodents, and not monkeys or man.

Summary of Part III.

1. Some experiments on the mechanism of the red-cell adhesion test are described.

2. In certain circumstances the washed red-cells of normal Primates adhere to trypanosomes; on the other hand, those of normal Ungulates, Felines, and Rodents apparently do not.

3. The red cells of a guinea-pig will adhere to the trypanosomes present in its blood if the plasma of a monkey suffering from chronic trypanosomiasis is added to the guinea-pig's blood; but if the red cells of a normal guinea-pig are

substituted for the red cells of the infected guinea-pig these normal guinea-pig cells will not adhere.

4. If the plasma of a guinea-pig, infected with trypanosomes and then sterilised by drugs, be mixed with the blood of another guinea-pig with an active infection, platelet and not red-cell adhesion will occur.

5. Reasons are given why we believe that red-cell adhesion and platelet adhesion are due to the same substance.

III. GENERAL DISCUSSION.

What then is to be learnt from these experiments? Hitherto investigators of the adhesion phenomenon have been unanimous in claiming that the reaction is strictly specific. Our experiments have shown that strong reactions may take place between reagents from sources differing widely in many important respects. Several explanations of this contradiction suggest themselves.

First, that red-blood-cell adhesion is of a different nature from platelet adhesion, the latter being strictly specific, the former fortuitous in its action. The experiments themselves, together with a number of controls carried out with proved sensitive trypanosomes and the blood of animals that have never been infected with trypanosomes, seem to justify the belief that the two manifestations are merely different expressions of the same physiological state. We have accordingly discarded this explanation.

Secondly, that the acutely pathogenic varieties of the well-known polymorphic trypanosomes found in many European laboratories react differently to the tissues of their hosts from the very much milder strains obtained from wild tsetse.

The interaction between parasite and host is, in the first case, so intense that the resulting adhesins, though sensitive, are very exacting in their requirements and operate only within very narrow and rigid limits. A relapse variant of one of these acute strains evokes no more response from the corresponding passage-strain plasma than do trypanosomes of a totally different species.

On the other hand, with the less virulent strains of tsetse-carried trypanosomes in monkeys and guinea-pigs there is a constant and fluctuating struggle going on between host and parasite, resulting, it may well be, in the formation of a number of different relapse variants, all present at the same time—variants of both the trypanosome and of the adhesin.

In these chronic infections race after race of trypanosomes occupies the blood in more or less rapid succession, according to the rapidity and extent of the output of antibodies by the host. Each round of this prolonged struggle adds to the total store of antibodies accumulated by the host, and leaves the blood in the partial or complete possession of a new race of survivors with its own peculiar serological attributes. Sterilisation of such an animal by drugs is equivalent to a hyper-intensive host reaction, leading to a sudden wholesale

destruction of trypanosomes and in consequence to a relatively enormous output of the particular antibodies corresponding to the trypanosome present at that particular moment.

If we assume that all the different antibodies evoked by the destruction of successive races of trypanosomes are preserved, at any rate for some weeks, in the blood of the host, then we can understand the otherwise puzzling catholicity shown by the blood of some of our chronically infected animals. As Brussin and Beletzky (1925) remark, with very virulent strains the multiplication of trypanosomes so greatly exceeds the restraining efforts of the host that no significant change in the race takes place, and the administration of a drug leads to a heavy production of a single type of antibody. Such a serum will be strictly "specific" or monovalent.

Partial reactions, we believe, are rightly explained by Rieckenberg and by Brussin and Beletzky as due to a mixed population of trypanosomes, some serologically homologous with the reacting blood, some not. This appears to us a more probable explanation than the quantitative theory of Johnson and Lester.

When we come to the interaction between reagents of apparently widely different origin such as *T. gambiense*, *T. rhodesiense* and the Damba strain, explanation is more difficult. This overlapping phenomenon is shown in our experiments by *T. gambiense*-monkeys 427, 513, 516, 544 and 612 and *T. rhodesiense*-monkey *515*. Monkeys 427, 516 and 544 reacted with *T. rhodesiense*, 513 with the Damba strain, and 612 feebly with *T. rhodesiense* and strongly with the Damba strain; *515* responded strongly on occasion with both *T. gambiense* and the Damba strain.

Such reactions could be explained in several ways. *First*, the adhesion of red cells may be merely a physico-chemical phenomenon without specific biological significance. This, for reasons already set forth, we do not believe. *Secondly*, our strains may have been mixed through carelessness or errors in technique. The system of supervision at the Entebbe Laboratory is directed mainly to the elimination of such accidents, which in work of this kind would of course be disastrous. The strains are under constant examination and remain true to type. Constant vigilance would certainly detect a mistake of this kind, yet hitherto we have had no cause for suspicion. Moreover the two *T. gambiense* monkeys whose blood shows best the response with *T. rhodesiense* trypanosomes have already lived far too long to allow of any suspicion of a double infection. *Thirdly*, that the reaction may be called forth by any member of the genus *Trypanosoma*. In refutation of this explanation we turn to our numerous negative tests and to the negative reactions given by *T. congolense* when exposed to the particular bloods under discussion. *Fourthly*, that these wild tsetse strains are not pure strains in the sense that the highly virulent strains of European laboratories may be considered pure. It is probable that animals and men living exposed to tsetse receive many bites from infected flies, and the strains so injected, of diverse origin, will be amalgamated into one complex

strain. In a region like the West Nile where there are two species of tsetse, game and a large scattered human population, this possibility must certainly be borne in mind.

And lastly, there remains the explanation that underlying the highly sensitive race adhesins there is another class of antibody corresponding roughly to the group agglutinins of bacteria.

Here we enter the realms of speculation and it is an intriguing vision that opens up before us. May not these overlapping reactions represent a group reaction embracing all trypanosome strains and species within a certain zone of zoological propinquity?

Several British workers have long held the opinion that *T. brucei* and *T. rhodesiense* are the same species of trypanosome. Kleine has recently maintained that *T. rhodesiense* is but an inexperienced *T. gambiense*, which finds itself in a new environment. And one of us has for years believed that *T. gambiense*, *T. rhodesiense* and the Damba strain, in fact all the tsetse-carried polymorphic trypanosomes, are fundamentally derivatives of *T. brucei*, and are still more or less plastic and sensitive to drastic changes in their environment.

The results we have obtained at all events suggest a new line of attack on an interesting problem in biology. It is hoped that fresh strains of *T. congolense*, *T. brucei* and *T. rhodesiense* will soon be available, with which to continue and extend the investigations now in progress.

IV. GENERAL SUMMARY.

The more important points that have emerged in the course of our investigations may now be assembled.

(1) An adhesion phenomenon has been described in which, in certain circumstances, red blood corpuscles adhere to trypanosomes.

(2) We believe this red-cell adhesion to be a manifestation of the adhesion phenomenon already described by other workers both with trypanosomes and spirochaetes.

(3) The substance responsible for this phenomenon of adhesion is designated in this paper *adhesin*.

(4) The red-cell adhesion test has been studied in relation to several strains of polymorphic trypanosomes, all of which were either recently isolated from naturally infected man or animals, or had been recently passed cyclically through tsetse.

(5) It is shown that the adhesion phenomenon is irregular and uncertain in its appearance. A single or even a number of negative observations do not exclude trypanosomiasis. A positive reaction, on the other hand, indicates recent or actual infection with a trypanosome of the same group, but not necessarily specifically identical with that employed as antigen in the test. Thus *T. gambiense* adhesin bloods not uncommonly react with *T. rhodesiense* trypanosomes and also on occasion with the Damba strain: the converse is apparently of less common occurrence.

(6) Adhesins appear in an animal's blood in the course of its infection with trypanosomes, whether the animal be treated with trypanocidal drugs or not. When sterilising doses of such drugs are employed the adhesins gradually disappear in the course of a few months; but in untreated infections of *T. gambiense* they have been shown to persist in an active form for more than two years.

(7) With our material it was found impossible to distinguish between "passage" and "relapse" strains by means of the adhesion test. On the contrary it was found, at all events with *T. gambiense*, that the best way to call forth the reaction in the blood of animals long infected is to employ trypanosomes from a very early infection.

(8) Guinea-pigs have been found to be unsatisfactory animals in which to demonstrate adhesins; they are, however, useful as a source of sensitive trypanosomes. When only guinea-pig elements are present in a test a positive reaction is indicated by platelet not by red-cell adhesion.

(9) The presence of adhesins in the blood has no apparent relation to immunity against the homologous trypanosome.

(10) The test has been applied to over 200 human cases of trypanosomiasis in the Uganda Protectorate, and with six of these repeated observations were made. Some of these human cases reacted both to *T. gambiense* and *T. rhodesiense*, but none to *T. rhodesiense* alone.

(11) A partial analysis has been made of the mechanism of red-cell adhesion, which has been shown to be in some way associated with the presence of the red corpuscles of primates. This fact probably explains why other workers appear not to have noticed red-cell adhesion in the extreme form we have described in this paper.

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THE OSCINIDAE (DIPTERA) AS VECTORS OF CONJUNCTIVITIS, AND THE ANATOMY OF THEIR MOUTH PARTS.

By G. S. GRAHAM-SMITH, M.D., F.R.S.

(With Plate XL, 1 Text-figure and 1 Chart.)

Some species of very small flies belonging to the family Oscinidae have been accused of transmitting conjunctivitis and other diseases in different parts of the world.

In the first part of this paper some of the evidence apparently incriminating these flies is quoted, and in the second part an account is given of the anatomy of the mouth parts, which appear to be so modified that the flies are likely to cause minute punctures when they feed on the delicate conjunctival epithelium or on recent wounds.

I. The transmission of conjunctivitis.

(1) *In India and the East.*

Perry and Castellani (1906) observed several cases of an unusual type of conjunctivitis during March, 1905, in Colombo.

"The outbreak was limited practically to the well-to-do classes....The onset of the disease was sudden, with early symptoms of an alarming nature, and its course was rapid. It yielded readily to treatment, and serious complications were rare. The Kock-Weeks bacillus (*B. ægyptiacum*) was obtained in cultures and smears. They failed to inject monkeys by instilling cultures into the uninjured conjunctiva, and conjunctival, subcutaneous and peritoneal inoculations produced no lesions in rabbits, guinea-pigs and mice. Acute conjunctivitis was, however, produced in a monkey by instilling a broth culture into the scarified conjunctiva."

They state that the weather conditions in March "favour the development of a small fly...popularly known as the 'eye-fly,'" but made no experiments with this insect.

Ayyar (1917) thought that *Siphunculina* (*Siphonella, Microneurum*) *funicola* de Meijere, 1905, the "eye-fly" of India, Ceylon and Java, was responsible for the spread of the severe forms of ophthalmia found in these countries. In many parts of India the fly is present throughout the year, except from November to February. "It is abundant during the summer months, and especially so during the short intervals of warm weather after the South West Monsoon sets in....In certain months of the year it is found a veritable nuisance to man." He notes that this fly has a special liking for lachrymal

secretions and sweat, though it feeds readily on exudates from wounds, and thinks it may be the carrier of "sore eye," a disease common amongst Indian children in the summer, and often attributed to eating too many mangoes."

Patton (1921), speaking of *Siphunculina funicola* in Madras, says that it is

"haematophagous in habit and that it is commonly seen on cattle and horses, where it follows the biting flies, feeding on the blood and serum which exudes from these bites, and also on the serous discharges from eyes, sores, etc....As is well known, the eye-fly can be an intolerable nuisance during the hot weather in the plains, as it has the habit of hovering in front of the eye and at the aural openings emitting a very characteristic buzzing sound. In the case of helpless children it settles on the inner canthus, and sucks up the fluid there; that it may transmit bacteria in this way can be easily understood. As it feeds on sores and the blood of animals it may easily infect the eyes, and it is more than probable that conjunctivitis and its sequelae may be acquired in the case of young children and weakly adults through its agency." (p. 603.) "It is extremely fond of collecting on sores, cuts, abrasions and the corners of the eyes especially of animals, feeding on the sanious or pustular fluid, which collects in those situations. I have seen large numbers on human sores, and if one cuts one's skin, this fly soon finds it out, and collects in a very short time and feeds on the blood." (p. 613.)

Fox (1921) showed that "Naga sore or Cachar boil which has long been known to be endemic in Assam, and in some years assumes the character of an epidemic" is associated with fusiform bacilli and spirochaetes similar to those found in Vincent's Disease. "In Assam an epidemic of Naga sore is associated with a plague of small flies...which swarm on the sores and mechanically convey infective material. Dr Hall Wright has actually infected himself with Naga sore through the agency of these flies. He has identified the fly as *Siphonella funicola*." During an outbreak in 1908 Patterson (1908) mentioned "the myriads of midge-like flies ('mango flies') that swarm on the exposed sores and are probably active agents on the dissemination of the infection." He stated that "*inoculation from man to man* failed on the healthy skin." On the other hand, if "a slight preliminary abrasion was allowed to partially heal, and the scab was then removed," and material from an ulcer placed on it a typical lesion developed. Roy (1928) states that this fly is a serious pest from May to June in Assam, and gives a chart showing that the seasonal prevalence of Naga sore and epidemic conjunctivitis coincide closely with the prevalence of the fly.

Roy states that the flies "are very active on a hot sunny day, but none can be found when the air is cool or the sky cloudy. They rest on the thatch, strings, and small strips of cane hanging down from the roof." It is very common in cow sheds, but a very few may be found in open fields. "They feed on warm human excretions, on the exudates of ulcers of man and of animals, putrid sloughs of ulcers, the discharge from the nostrils or eyes, and from the rectum of cattle, and cow and horse dung, fresh and decomposed."

Senior-White (1923), who studied the anatomy of the proboscis, thought the fly was unable to pierce the unbroken skin, but used the projecting free ends of the pseudotracheal rings as scarifying organs to remove dried crusts

and reach the serous exudations beneath them. Roy (1928), however, says that the fly settles on the healthy skin surface at the margin of an ulcer and tries to force its proboscis through a rent in the scab to get at the exudation below.

Ayyar (1917) and Patton (1921) failed to find the natural breeding places, but the former bred it on fresh cow dung, and noted that in June the period from egg to adult averaged 18 days. Hutson (1927) reports that Jepson and Pinto were also unable to find the natural breeding places, but observed the entire development of *S. funicola* on moistened blood meal, and also bred them

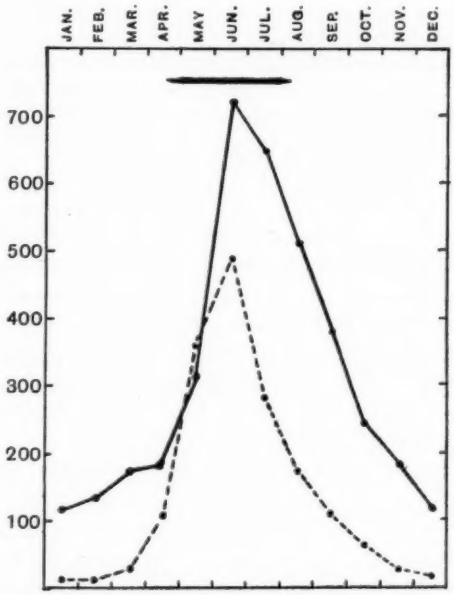


Chart showing incidence of Naga sore (continuous curve) and epidemic conjunctivitis (interrupted curve) in a tea estate in Assam during 1924–5. The horizontal line above shows the period during which *Siphunculina funicola* is a serious pest. Redrawn from the chart given by Roy (1928).

in decomposing animal and vegetable matter. In the open the generations follow each other in about 20 days. Roy (1928), in Assam, bred them from earth sodden with dung and urine in cattle sheds, but did not obtain them in traps placed over human faeces, cow or horse dung, decaying fruit, leaves, etc.

(2) *In Egypt.*

Towards the end of the year flies belonging to this family are very common in some parts of Egypt and their relation to the prevailing ophthalmia should be investigated.

Cottam (1923) states that

from September to December, *Oscinis pallipes* Lamb, causes considerable annoyance to householders in certain parts of Khartoum by its habit of assembling in dense swarms or clusters on ceilings and upper parts of walls of rooms and verandahs. These assemblies, which consist of countless myriads of flies of both sexes, the females predominating, may occur in either the ground or upper stories, and appear in precisely the same places year after year...the shady and sheltered situations are favoured by the flies....A cluster of these flies, of average size, covers an area of approximately a square yard, but others covering twice or even three times that area may be met with....If one of these swarms is undisturbed it will persist for several weeks, while if destroyed another will collect in the course of a few days to take its place. A fairly loud humming noise is made by the flies when disturbed. It is probable that these flies assemble for mating purposes, and that none of them spend more than a day or two in the cluster....

I have bred *Oscinis pallipes* in all stages in the laboratory on decaying vegetable matter such as rotting grass, rotting "dura," leaf mould, and horse and cow dung, but have not yet found the immature stages in the field, although considerable material of the above nature has been collected and carefully examined. Under laboratory conditions the life cycle of the fly occupied from six to fourteen days....

Although the economic importance of this fly is so far not fully known, nevertheless it may be classed as a pest, for the clusters are unsightly and the flies stain the walls and ceilings with their excrement. Furthermore, they are attracted to artificial light at night and constitute a nuisance by swarming round lamps or on dinner tables and getting into people's eyes.

Lamb, in the same paper, gives a detailed description of the species under the name *Oscinis* (*Oscinella*) *pallipes* n.sp. "They are characterised by their entirely shining black colour, with the antennae red, or at least reddish, and blackened on the end of the third joint, while the legs are almost wholly pale." Length, 1·6 mm.; wing, 1·5 mm.

Chloropisca circumdata has the same habit, but the swarms are much smaller.

(3) *In the United States.*

Herms (1926) describes the trouble in the Coachella Valley, California, due to a small fly, *Hippelates pusio* Lw., originally described as *flavipes*, Lw., belonging to the family *Chloropidae* (*Oscinidae*). "It measures about two millimetres in length, is active from daylight to dark from nine to ten months in the year....It is a most persistent little creature buzzing around the head, settling momentarily on one's clothing, darting frequently at the eyes and into the ears. Drops of blood on the skin, open wounds, sores, mucous membranes and so forth are particularly attractive to the flies." It hovers round animals in the same way.

The Coachella Valley, which borders upon the Salton Sea,

lies on an average of about 125 feet below sea level and has a population of about seven thousand inhabitants....During the last ten or more years *Hippelates* flies have become increasingly numerous, until now they are a veritable pest and together with numerous cases of 'pink eye' affecting the people of the valley and the known relation of the fly to the disease, there exists a really serious situation....Workmen, both in the field and shop, are very seriously hampered in their duties and animals suffer greatly. Fruit pickers are

much hampered, pollinating dates is made difficult, school teachers are hard to keep, workmen in general will not stay on the job, absences among school children due to pink eye mount up.

"Pink eye" is a catarrhal conjunctivitis most frequent in children, though adults are frequently affected.

During the next three years a great increase in the numbers of *H. pusio* was observed, and at Thermal, California, 15,000 school children were notified as suffering from "pink eye" (*Science*, 1929, p. xiv). "Florida is also having some trouble with pink eye." Though various baits have proved attractive, oviposition has not been observed, and its breeding places are unknown (Herms, 1928).

(4) *In the West Indies.*

Nicholls (1912), working in St Lucia, Windward Islands, made the following statement: "I believe that the majority of cases of yaws (framboesia) in the West Indies are caused by the inoculation of surface injuries" by *Hippelates flavipes*, Lw. (originally called by him *Oscinis pallipes* Lw., but see Aldrich (1905)).

They feed only on the skin discharges of man and other animals, and though rare in the town of Castries, they are very numerous in the country districts of St Lucia, and can be seen hovering round the bare legs and arms of labourers, searching for abrasions or the secretions of the sweat and sebaceous glands. The persistence of these little flies is extraordinary; they must be brushed off by actually touching them, and they will immediately return. If undisturbed, they engorge themselves with pus, blood, serum or sebaceous secretion, until their abdomens are greatly distended.

(5) *In Europe.*

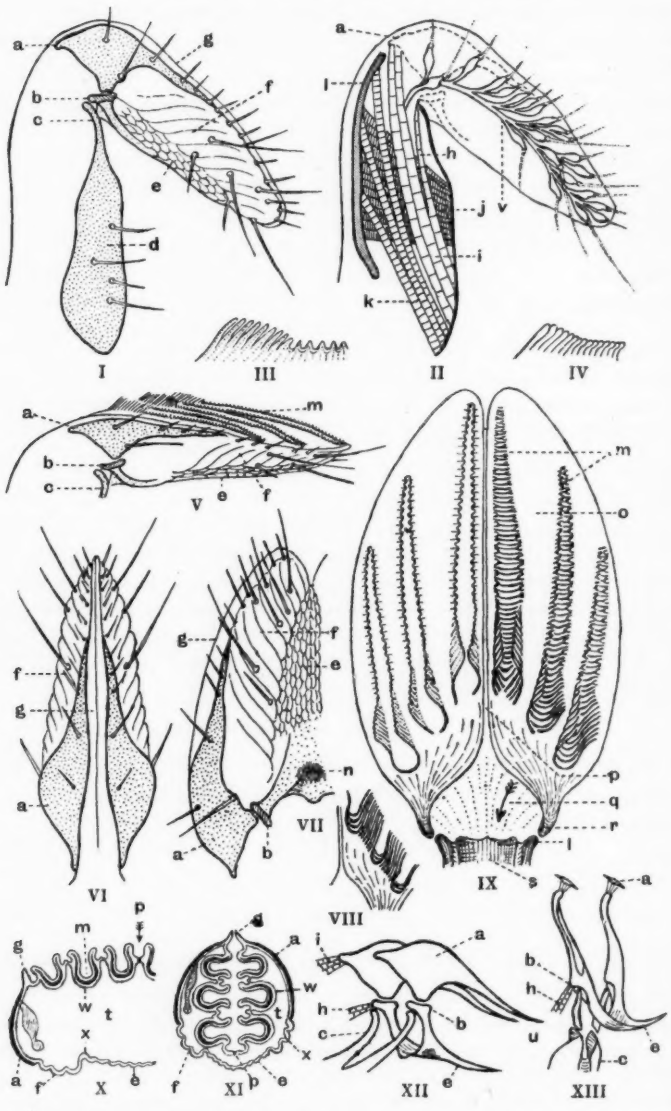
In England, Germany and elsewhere, some species occasionally occur in rooms in considerable numbers, but there is no evidence that they cause serious annoyance or transmit disease.

II. THE ANATOMY OF THE MOUTH PARTS.

(1) *Siphunculina funicola.*

Senior-White (1923) describes the anatomy of the proboscis of the Oriental "eye-fly" as follows:

Though completely retractile, the proboscis of *Siphunculina funicola* can be seen at a glance to have undergone considerable modification from the Muscoid type. The rostrum is enormously developed... while the labella are greatly narrowed and nearly as long as the haustellum. Minute examination reveals that the rostrum and haustellum show no essential departure from the normal *Musca* type... but the furca and discal sclerite are well developed, and, with the lateral margins of the labella, strongly chitinised, whilst the pseudotracheal channels are reduced in number to six, without collecting channels, all opening directly at the prestomum. The basal rings of the four inner channels are separated at their ends much as illustrated by Cragg for *Musca inferior* Stein... but here development has been carried much farther. There is no trace of the teeth or interdental armature of the biting *Musca*, but the place of the latter is taken by the free ends of the pseudotracheal rings,



Text-fig. 1. All the drawings refer to *Hippelates pusio* except VIII and XIII. I. Side view of the haustellum and oral disc in the resting position. II. Median longitudinal section of the same, showing the disposition of the muscles in the haustellum and the distribution of the labial nerve to the end-organs of the chaetae in the labellum. III. Side view of the proximal part of a pseudotrachea, showing the relationship of the integument to the chitinous rings (dotted). IV. The appearance of III, when viewed obliquely. V. Side view of the oral disc in the feeding position to illustrate the disposition of the "spines" formed by the terminations of the

some of which are enormously elongated with spines which rise above the inner surface of the labella, brush-like. The rotation of the labella on the discal sclerite must draw those spines outwards just as the teeth of *Musca* are drawn, and though their strength and chitinisation would not appear sufficient to enable them to pierce unbroken skin, they doubtless act as scarifying organs, enabling the fly to remove dried scab to reach serous exudations below.

He illustrates the outlines of the proboscis and the chitin of the pseudotracheal rings.

Manson (1914) states that a breach of surface is necessary for infection with yaws, and it is well to know that the Oriental "eye-fly" at least is provided with a biting apparatus sufficient to pierce the dried surface exudations of a pre-existing ulcer, where the same authority states that the disease often originates. (Senior-White, 1923.)

(2) *Hippelates pusio* Lw.

Through the kindness of Prof. Herms the writer was supplied with a large number of specimens of *H. pusio* from the Coachella Valley which had been fixed in Bouin's solution, and was therefore able to make a careful study of the anatomy of the mouth parts.

The haustellum is relatively large, but resembles in structure that of *C. erythrocephala*. The cornua of the mentum[1] are very long and appear to articulate with tubercles on the lateral processes of the furca without the intervention of mento-furcal bars, which do not seem to be represented. The paraphyses are strongly developed and the labial gutter, which is relatively very wide, is roofed over proximally by the large labrum-epipharynx and distally by well-developed disco-haustellar folds. Within the haustellum the

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pseudotracheal rings. VI. Dorsal view of the oral disc in the resting position, showing the marginal strips, supported by the epifurcas, meeting along the prestomal margin. VII. Ventral view of half the oral disc in the feeding position. VIII. A portion of the inner surface of the right labellum of *H. flavipes*, showing spines (interdental armature) between the openings of the pseudotracheae. IX. The inner surfaces of the labella in the feeding position. On the right the rings of the three pseudotracheae are shown and on the left the disposition of the integument in relation to the pseudotracheae. X. Transverse section through half the oral disc in the feeding position (in its proximal third). XI. Transverse section through the same region in the resting position. XII. The furca, the epifurcas and the cornua of the mentum. XIII. The same structures in *C. erythrocephala* for comparison with XII. *a*, epifurca; *b*, tubercle of lateral process of furca; *c*, cornu of mentum; *d*, mentum; *e*, ventral surface of oral disc covered with small scales and supported by the median process of the furca; *f*, outer wall of labellum showing prominent folds; *g*, marginal strip; *h*, retractor muscle of furca; *i*, retractor muscle of epifurca; *j*, transverse muscle of haustellum; *k*, retractor muscle of paraphysis; *l*, paraphysis; *m*, pseudotrachea; *n*, pigmented area of "spring" of furca; *o*, inner wall of labellum; *p*, syndesmosis between the labella; *q*, thin membrane passing from the discal sclerite to the hypoglossa and paraphyses and forming the floor of the oral aperture (arrow); *r*, strongly chitinised process of the discal sclerite articulating with the head of the paraphysis; *s*, hypoglossa; *t*, haemocoel between the inner and outer walls of the labella; *u*, mento-furcal bar; *v*, labellar portion of labial nerve and its branches to the end-organs of the chaetae (dotted); *w*, sub-pseudotracheal membrane; *x*, hypodermis lining the outer wall of the labellum.

[1] For the nomenclature used see Graham-Smith (1930).

same muscles occur as in *C. erythrocephala*, but the retractor of the furca is divided into two portions, a smaller, which is inserted into the tubercle of the lateral process of the furca, and a very strong portion, which is inserted into the proximal angle and adjacent side of the enormous epifurca, and may therefore be termed the retractor of the epifurca.

The oral disc is much modified, the portion corresponding to the posterior half in *C. erythrocephala* only being present.

On the external wall of the labellum the most conspicuous object is the very large, darkly pigmented epifurca, forming a triangular plate, whose distal angle extends to a point beyond the centre of the free margin of the prestomum. Its proximal angle is strong and pointed and into it and the adjacent proximal side the retractor of the epifurca is inserted. The furcal angle articulates with a facet on the lateral process of the furca. The epifurca bears several chaetae and the marginal strip springs from its distal border. Whereas in comparison with the parts in *C. erythrocephala* the epifurca is very large, the furca is small, and consists of two lateral processes joined together by a broad, thin "spring," somewhat pigmented at its centre, from which the median process originates. The lateral process terminates in a strong transverse bar, the proximal end of which corresponds to the tubercle in *C. erythrocephala*, and into it the retractor of the furca is inserted. The distal end corresponds to the portion of the lateral process which, in *C. erythrocephala*, lies beyond the tubercle, and with it the epifurca articulates. The central process forms the flat, elongated, triangular base, from which the outer walls of the labella spring, covered except in its median proximal portion, which is smooth and pigmented, with small, oval scales.

The outer walls of the labella, arising from the central process of the furca, pass to the prestomal margin and terminate, as in *C. erythrocephala*, in thin marginal strips, which are in apposition in the resting position. The outer walls bear numerous chaetae and exhibit several oblique folds, which are particularly marked near the central process.

The inner wall of each labellum consists of a thin sheet of chitin, grooved by three pseudotracheae, the inner running nearly longitudinally, and the outer two diverging towards the prestomal margin. The proximal portion of the inner wall is more strongly chitinised and terminates in a curved process, representing the bar of the discal sclerite, which articulates with the distal end of the paraphysis. The two labella articulate by a syndesmosis along the mid-line of the oral disc, except proximally where they separate to form the bars of the discal sclerite, and enclose the oral aperture. From these bars a thin membrane, forming the ventral wall of the oral aperture, passes to the distal end of the hypoglossa.

The pseudotracheal membrane, namely, the part of the inner wall which bears the pseudotracheae, shows no evidence of being divided into definite pseudotracheal and inter-pseudotracheal plates.

Within the labellum the labial nerve passes forwards near the base of the

outer wall, sending branches to the large end-organs in connection with the chaetae, but there is no large branch to the inner wall and no gustatory papillae are visible, either between the pseudotracheae or in relation to their distal extremities. The sub-pseudotracheal membrane is relatively loose under the pseudotracheae, but between them is closely connected with the pseudotracheal membrane. The hypodermis is closely attached to the outer wall. No evidence of the presence of tendinous cords was obtained.

The pseudotracheae, which appear to remain permanently and widely open along their central lines, increase slightly in width from their distal to their proximal ends. Each channel is supported by about 60 chitinous rings, which, except near its proximal end, have alternate flattened and slightly bifid extremities. The proximal 10 to 15 rings terminate in sharp spines, the longest proximally. In all regions of the pseudotrachea the extremities of the rings turn outwards and project above the general surface of the labellum, instead of turning inwards and covering the pseudotracheal channel. The extremities are not, however, naked, as Senior-White appears to think is the case in *S. funicola*, but are covered by the integument, which binds together the spines of the proximal rings so that they form a deeply serrated saw-edge parallel to the pseudotrachea; and distally binds, as in *C. erythrocephala*, the flattened extremities of the intermediate rings to the extremities of the adjacent forks. The latter arrangement produces along the greater part of the pseudotrachea a series of sharp ridges nearly at right angles to its length. Raised, sharp cutting edges are therefore provided, whether the labella are moved laterally or longitudinally. In the feeding position the pseudotracheal surface resembles a curry-comb.

The proximal portions of the pseudotracheae, which are provided with elongated spines, correspond to the central collecting channels of *C. erythrocephala*, but neither common collecting channels nor prestomal teeth are present.

In the resting position the inner walls of the labella, hinging along the central syndesmosis, are in apposition (Text-fig. 1, xi), but in the feeding position the epifurcas and the lateral processes of the furca are drawn upwards and outwards by the action of their retractors and the pseudotracheal surfaces of the two labella are thus brought into one plane (Text-fig. 1, x). In this position the oral aperture is widely open, and the pseudotracheae, which also seem to be open and consequently incapable of performing the function of filtering, act as channels leading towards it. It seems, therefore, that a "direct feeding position" in which large particles can pass into the oral aperture, is always employed. In fact the study of the mouth parts suggests that, when the fly is feeding, the tips of the pseudotracheal rings act as cutting or scarifying instruments, capable of causing minute multiple incisions in granulation tissue or the delicate conjunctural epithelium, which are likely to assist pathogenic organisms carried by the fly in gaining a foothold.

(3) *Hippelates flavipes* Lw.

Specimens, preserved in alcohol, sent some years ago by Dr Nicholls from St Lucia were examined. In most respects the mouth parts are very similar to those of *H. pusio*, but the lateral processes of the furca are stronger. The six pseudotracheae are constructed and arranged in the same manner, but a number of delicate spines, presumably corresponding to the prestomal teeth (interdental armature) arise between their proximal terminations. They form additional cutting instruments. (Text-fig. 1, VIII.)

(4) *Oscinis pallipes* Lamb.

Some dried specimens from Khartoum, kindly given by Dr Lamb, were examined. The mouth parts appear to resemble those of *H. pusio* very closely.

Conclusions.

In several parts of the world, including India, Java, California and the West Indies, different species of very small flies belonging to the family *Oscinidae* appear to transmit conjunctivitis and possibly other diseases.

In all the species examined the mouth parts are very similar. In each there are six pseudotracheae with the tips of their chitinous rings upturned to form spines, covered by the integument, projecting above the level of the pseudotracheal membrane. When the flies are feeding on abrasions or the conjunctival epithelium, these spines apparently act as cutting instruments capable of producing minute multiple incisions, likely to assist pathogenic organisms carried by the insects in gaining a foothold.

Allied species, which have similar habits and possess mouth parts of similar structure, are likely to act as vectors of pathogenic organisms to men and animals.

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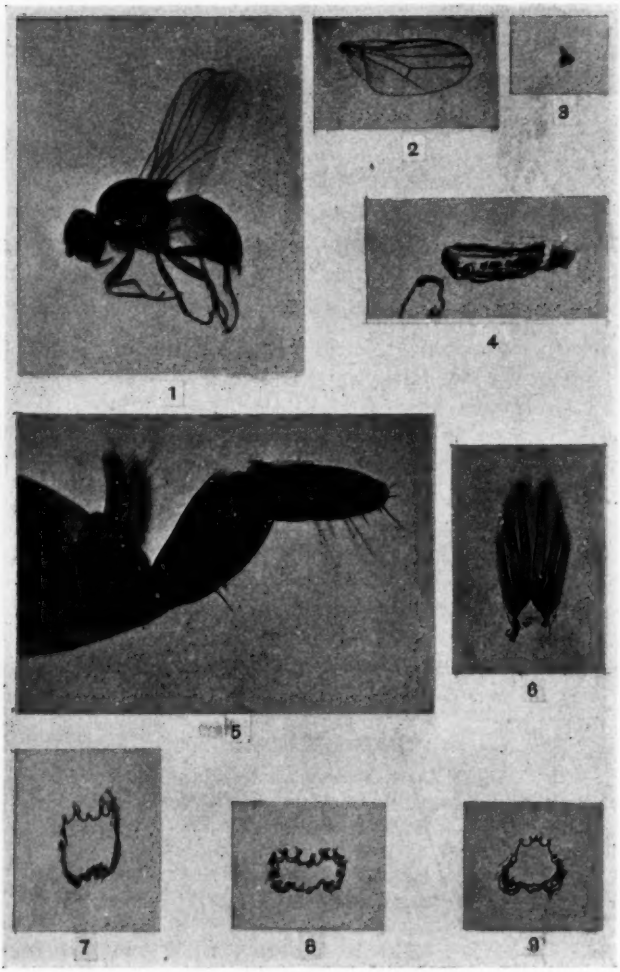
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EXPLANATION OF PLATE XL.

Fig. 1. *Hippelates pusio* (×11), side view. Fig. 2. Wing. Fig. 3. Natural size. Fig. 4. Longitudinal section of the labellum through the outer pseudotrachea, immediately below which is seen the sub-pseudotracheal membrane. The dark masses below the latter are the end-organs of the chaetae. Fig. 5. Side view of part of the rostrum, the haustellum and oral disc in the feeding position (×90). The spines of the proximal rings of the pseudotracheae are seen projecting upwards for about one-third the length of the labellum. Beyond them the upturned ends of the distal rings give the appearance of a fine saw-edge. Fig. 6. The labella (×90) spread out in the feeding position, showing the positions of the six pseudotracheae, the discal sclerite and the oral aperture. Fig. 7. Transverse section of the disc in the feeding position immediately beyond the opening of the inner pseudotracheae, showing spines, covered by integument, projecting from the outer sides of these channels. Fig. 8. Transverse section of the same disc near its centre (see Text-fig. 1, x). Fig. 9. Transverse section of the same disc beyond the terminations of the outer pseudotracheae.

For these photographs I am indebted to the care and skill of Mr H. P. Hudson, of the Pathological Department, Cambridge.

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CHONDRACANTHUS NEALI N.SP., A PARASITIC COPEPOD OF *MALACOCEPHALUS LAEVIS*.

By W. H. LEIGH-SHARPE, M.Sc. (Lond.).

(With 2 Text-figures.)

Habitat and material. By the courtesy of Prof. W. M. Tattersall of University College, Cardiff, I have been able to examine what proved to be a new species of *Chondracanthus*, the material consisting of a female with a male attached anterior to the abdomen as is usual. The host, *Malacocephalus laevis*, was taken in deep water from S.W. Ireland by the fishing fleet of M. H. Neale, Esq., the eminent Cardiff naturalist, after whom this species is named.

The new species is interesting as standing midway between *C. zei* and *C. lophii*, being profusely ornamented with processes like *C. zei*, while the male most closely resembles that of *C. lophii*.

The Female.

Body. The outline of the female's body can be best seen from Fig. 1. Total length 9 mm. The *cephalothorax* is large and spherical, one thoracic segment forming a short but well-marked neck being fused with the cephalon. The mouth is well on the ventral surface of the cephalon. The cephalothorax thus far resembles that of *C. zei*. The *first antennae* are large, and stand out laterally and conspicuously at right angles to the cephalon. Behind them and protruding still farther laterally at right angles to the cephalon are new structures, which I will here define as the *cephalic horns*, and which, as far as I can ascertain, occur only in one other species, viz. *C. distortus* Wilson. Behind the cephalic horns, protruding laterally in the same manner and nearly twice as long, are the *cephalic barbs*[1]. The *dorsal processes*[1] are long and stout. There are three pairs of long bifurcated lateral processes[1], and a pair of long posterior processes[1].

The *trunk* is considerably longer than wide, and terminates in a small, elongated, median process bearing a male. The *pereiopods* (ventral thoracic legs) are two pairs and bipartite. There are three median dorsal lobes (not four as in *C. zei*), elongated (not spherical tubercles as in *Disphaerocephalus*), but resembling those of *C. lophii*. The majority of the species are without these lobes and lateral processes.

[1] These terms are used in the sense defined by Oakley for the genus *Chondracanthus* (*Parasitology*, **22**, 186).

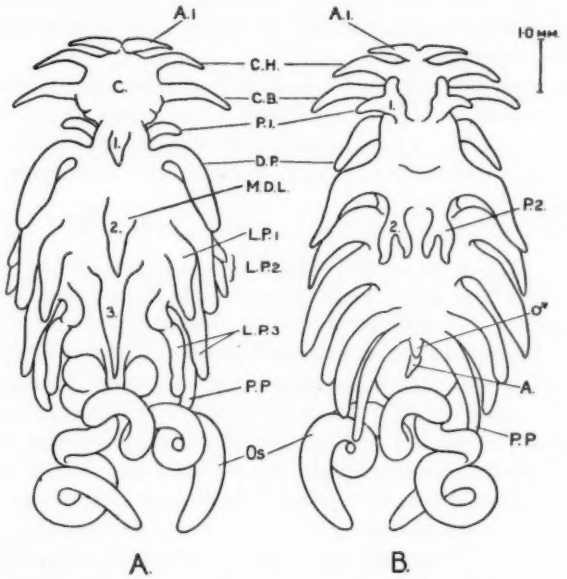

Fig. 1. *Chondracanthus neali* n.sp. ♀. A. In dorsal aspect. B. In ventral aspect. *C.* = cephalothorax; *A.* 1. = first antenna; *C.H.* = cephalic horns; *C.B.* = cephalic barbs; *D.P.* = dorsal processes; *L.P.* 1. 2. 3. = lateral processes; *M.D.L.* 1. 2. 3. = median dorsal lobes; *Os.* = ovisacs; *P.P.* = posterior processes; *P.* 1., *P.* 2. = first and second pereiopods; *A.* = abdomen; ♂ = male

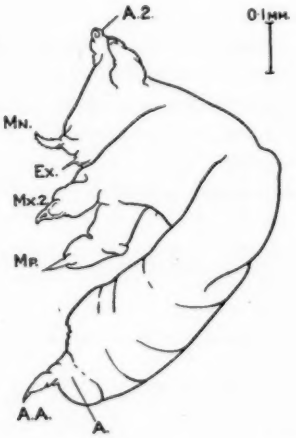

Fig. 2. *Chondracanthus neali* n.sp. ♂. *A.* 2. = second antenna; *Mn.* = mandible; *Ex.* = exopodite of mandible; *Mx.* 2. = second maxilla; *Mp.* = maxillipede; *A.* = abdomen; *A.A.* = abdominal appendage.

A small reduced *abdomen* succeeds the terminal process of the trunk. That the abdomen in the Chondracanthidae is always undivided was first pointed out by Oakley (*Parasitology*, **19**, 461).

The *ovisacs* are long and much convoluted; this does not occur in any other species of *Chondracanthus*. They are bright yellow, while the animal is translucent white.

As the type is desired for a museum specimen it is not possible to examine further the anterior appendages.

The Male.

The outline of the male's body is seen in left lateral aspect in Fig. 2. It resembles that of *C. lophii* in having an elongated posterior trunk region, which in the present case is much longer than in any hitherto described male. Total length 0·75 mm. The appendages closely resemble other males of this uniform genus. I am unable to make out the first antennae. Second antennae conspicuous and terminally hooked. Mandibles falciform and toothed; the exopodite (or palp) with a pronounced terminal seta. Second maxilla two-articled, but the usual seta on the basal article is lacking, at any rate in this specimen. The maxillipede closely resembles that of other species. The pereiopods (thoracic legs), two pairs, were visible before mounting, but cannot be made out in a Canada balsam mount, owing to its great transparency. On the other hand, the posterior trunk showed no trace of segmentation before mounting, and only very slight indications afterwards, due possibly to shrinkage. The abdomen is large in proportion, not well marked off from the trunk, and bearing terminally the usual pair of slender unjointed abdominal appendages.

Specific characters. Female. Cephalic horns present anterior to barbs. Pereiopods bipartite. Dorsal processes one pair. Three pairs of lateral trunk processes bifurcated. Three elongated median dorsal lobes. Cephalothorax large and spherical. Egg-strings long and much convoluted.

Host. *Malacocephalus laevis*.

Male. Posterior trunk considerably elongated.

(*MS. received for publication* 27. v. 30.—Ed.)

STUDIES ON SOME SPOROZOA IN POLYCHAETE WORMS.

II. *DORISIELLA SCOLELEPIDIS*, N.GEN., N.SP.

By HARENDRA NATH RAY, Ph.D. (Lond.),

Guruprasanna Ghose Scholar, University of Calcutta.

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(With Plates XLI and XLII.)

Among the Sporozoa inhabiting the gut of *Scolelepis fuliginosa* Claparède at Plymouth, I have often found an organism that I believe to be a peculiar coccidian, although certain important stages in the sexual part of the life cycle have hitherto escaped me. If I am right in my opinion, then this sporozoan is a new form, belonging to no genus of coccidian hitherto described: I propose for it the name *Dorisiella scolelepidis*.

Coccidia seem to be very rare parasites of annelid worms. The only species that have been studied in any detail are *Caryotropha mesnilii* Siedlecki (1907), *Angeiocystis audouiniae* Brasil (1909) and *Myriaspora trophoniae* Lermantoff (1913): some observations have also been published (1930) by Dehorne and by Thomas on a coccidian which the former author regards as a species of Aggregata and the latter as probably more nearly related to *Caryotropha*. The first of these lives in the bundles of developing spermatozoa in the coelom, the second parasitises the tissues of the "heart," the third occurs in the body cavity, and the fourth in the body cavity, nervous system, etc. Only in *Caryotropha* is the schizogonic portion of the life cycle known.

The organism I am dealing with here is the first gut-inhabiting organism remotely resembling Coccidia that has ever been described in detail. In 1897 Mesnil and Caullery recorded from the gut epithelium of *Capitella capitata* parasitic organisms which they were then disposed to regard as coccidian in nature, though in a later paper (1901) they departed from that view. Cecconi (1905) again saw these Sporozoa in *Capitella* and thought them possibly coccidian, but the pictures he gives tell one nothing.

MATERIAL AND METHODS.

The worms came from Plymouth and my observations were made over a period of 18 months. I examined many at Plymouth, and while in London I also kept living worms under aeration with frequent change of sea-water, in which they remained quite healthy.

In order to observe the parasite in the living condition the worm was cut into several pieces: each piece was then teased out separately on a slide and examined under the microscope. As far as possible, the parasite was examined in the fluid medium of the worm which occasionally was supplemented with sea-water.

Fixed and stained preparations, smears as well as serial sections, were made for studying in detail the life history of the parasite. Smears were fixed in Schaudinn's fluid and a modification of Bouin's fluid[1]. Fifteen minutes to 1 hour's fixation was found to be quite satisfactory for the smears. The same fixatives, as well as Flemming's fluid (both weak and strong) and Zenker's fluid were used for the material to be embedded. Here I found Bouin's fluid by far the most satisfactory. The small size of Scolelepis makes it extremely difficult to dissect out the alimentary canal entire; hence it was found best to cut the worm transversely into small pieces, which were separately embedded and sectioned. 75 per cent. alcohol containing a small quantity of lithium carbonate was used to wash out the picric acid from the material fixed in Bouin's fluid: immersion of smears for 30 minutes to 1 hour, and of the tissues for 2 to 6 hours in this medium removes from them the visible traces of picric acid without causing any apparent injury.

Sections 6μ–8μ thick were found to be very useful. The observations recorded here are mainly based on material fixed in the modification of Bouin's fluid and stained either in iron haematoxylin or in Dobell's modification of Mann's methyl-blue-eosin. Certain "cytoplasmic" fixatives, such as Cajal alcohol and Da Fano's and Helly's fluids, were also used. It was only with the last-named fluid that some success was obtained; such sections were then stained in Altmann's acid fuchsin and toludin blue differentiated with aurantin, and in iron haematoxylin.

THE LIFE CYCLE OF *DORISIELLA SCOLELEPIDIS*.

I cut and examined serial sections of 250 worms, and I found that about 75 per cent. of the animals were infected. The infection is usually confined to the middle and posterior portions of the digestive tract, and the greater part of the life cycle is passed in the epithelial cells. The gut epithelium along the first and last 25 to 30 segments of the body is free from the parasite; this perhaps is due to the greater thickness of the outer face of the cells in these two regions. Although the hosts look quite normal and healthy, the infected cells appear to suffer a good deal and become vacuolated as their cytoplasm is used up by the growing parasite; relatively extensive areas may be thus affected. I may point out here that the simultaneous occurrence of other gut parasites—Sporozoa (Polyrhabdina, two species of *Selenidium* and a sub-epithelial parasite of unknown affinity), a parasitic dinoflagellate and an

[1] Sat. picric acid in 90 % alcohol ... ... 2 parts
Sat. mercury bichloride in distilled water ... 3 parts
Formalin ... ... 1 part
Gl. acetic acid ... ... 2 parts

astomatous ciliate—added to the difficulties of deciphering the stages of the life cycle of the coccidian. But, fortunately, each of these organisms has certain morphological characters of its own which enabled me to distinguish one from the other readily enough after a little practice. One must also be on one's guard against interpreting as stages of this parasite small masses of organic material in vacuoles, which the epithelial cells often contain.

(*a*) SCHIZOGONY.

(i) *Macroschizogony.*

I will begin my description with the intracellular schizont, which I believe to have developed from a sporozoite escaped from an ingested spore. A young schizont (the small individuals in Pl. XLI, fig. 1) is about 12μ–14μ long and 4μ–6μ broad, which dimensions correspond approximately to those of a sporozoite, except that the latter is only 2μ–4μ broad; this slight change in diameter is perhaps the result of nutrition. There are two chromatic granules at its anterior end. The nucleus at this stage has no central karyosome[1], and shows a peripheral arrangement of the chromatin, with one or two large "blobs" of chromatic substance placed at either pole, whereas a sporozoite shows only one chromatic extra-nuclear dot and has a small karyosome. The change in the nucleus of the young schizont may be effected by ejection into the cytoplasm of the karyosome of the sporozoite (see below) and its conversion there into the second chromatic dot of the schizont. As the schizont grows, a tangled skein forms in its nucleus and the chromatic granules at its anterior end increase in number; finally there are five to six such granules and the schizont is 20μ–26μ long and 6μ–10μ broad (Pl. XLI, fig. 2). As I have seen the stages in which the chromatic blobs at the periphery of the nucleus seem to be emerging into the cytoplasm, and as I have never seen the intracytoplasmic granules dividing, I am rather inclined to think that these chromatic granules result from the activity of the nucleus. Of their chemical nature or function I know nothing. Like the blobs in the nucleus they stain black with iron haematoxylin and pink with Mann's methyl-blue-eosin: stained with Altmann's stain after fixation with Helly's fluid they appear red. Comparable structures are known in the coccidian *Angeiocystis audouiniae* and in the species of *Selenidium* I have described (Ray, 1930). But it should be noted that the chromatic bodies in my coccidian are never thread-like as they are in the gregarines living alongside it in *Scolelepis*.

It may also be observed that the schizonts quite as often lie with their anterior ends towards the lumen of the gut as towards the bases of the epithelial cells. This is in marked contrast to what happens in *Selenidium* (Ray, 1930), which, in the intracellular stage, almost always has its anterior end downwards, and has to back out from the cell in order to escape into the gut.

[1] Throughout this paper "karyosome" is used for the dark-staining spherical body in the nucleus, and I prefer not to commit myself to speculation as to its exact nature.

Now the schizogony begins: a spindle is formed, usually parallel to the long axis of the body (Pl. XLI, figs. 2–3) and centrosomes can sometimes be seen lying in the cytoplasm. An equatorial plate is formed (Pl. XLI, fig. 4). At the anaphase a group of curved chromosomes is seen lying at either pole (Pl. XLI, fig. 5). I have tried to count the chromosomes, but this stage is very rare in my preparations and I am not quite certain whether the number is three or four. At this stage the chromatic blob from the nucleus is attached to the spindle. It is probably cast out or degenerates, for there is no evidence of its being divided and carried over to the daughter nuclei. Similar chromatic blobs reappear in the daughter nuclei prior to division (Pl. XLI, fig. 6). This process is repeated a number of times, until finally there are 40 to 50 nuclei and the schizont has attained the dimensions of 40μ–$45\mu \times 12\mu$–15μ (Pl. XLI, fig. 7). At this stage each nucleus is accompanied by a chromatic granule in the cytoplasm. Just when the nuclei are all formed (the number varies between 40 and 50) I notice that a chromatic blob lies at the periphery of each, and I assume that it is this blob, or part of it, that is extruded and becomes the intracytoplasmic chromatic dot. With the differentiation of the merozoites as spindle-shaped bodies (Pl. XLI, fig. 8) one end of the schizont increases considerably in diameter, while the other end becomes shrivelled and vacuolated, and here the degenerating granules of the parent can sometimes be detected. At this stage a group of merozoites looks like a "hand" of 40 or 50 bananas, five to eight rows deep, the shrivelled end of the old schizont acting as the base for their attachment. A merozoite is pointed at both ends and is about 12μ–16μ long and 2μ–4μ broad (Pl. XLI, figs. 8 and 9); its nucleus is situated slightly towards its posterior end and shows a minute karyosome, and there are now two chromatic granules, placed one behind the other, between the nucleus and the anterior extremity. Where the second chromatic dot has come from I do not know. The group of merozoites now bursts and the liberated merozoites attack fresh epithelial cells either directly or fall into the lumen of the gut, whence presumably they invade other cells (Pl. XLI, fig. 9). I can bring no direct evidence to show that there is more than one generation of these macro-merozoites, but the infection of the gut epithelium is so intense that I think it probable.

The schizogony of *Dorisiella scolelepidis* was actually seen by Caullery and Mesnil (1901) when they were studying the "schizogregarines," *Selenidium* spp., that live in company with it; but these observers concluded, on too slight evidence, that they were dealing with the asexual phase of one of the gregarines. "La parasite intracellulaire, d'abord en forme de croissant, prend peu à peu la forme sphérique; en même temps son noyau se multiple, la sphère se resout en un barillet schématique de 7–8μ de hauteur et composé d'une douzaine[1] d'éléments avec un petit reliquat polaire. Les mérozoites ainsi

[1] I think this number is incorrect. I find that the ordinary schizogony gives rise to 40 or 50 merozoites about 12μ in length. In the development of micromerozoites (see p. 475), the number in a group is eight; the size of these, about 8μ–10μ in length, agrees better with Caullery and Mesnil's observation.

formés se séparent, tombent dans la luminaire de l'intestine, s'accolent par la pointe aux cellules intestinales et croissent en restant extracellulaire. Nous avons suivi cette évolution *en détail et sans lacunes*[1]. Cette observation confirme l'existence de la schizogonie intracellulaire dans cette group des Grégarines."

That other Sporozoa might occur along with the schizogregarines does not seem to have occurred to Caullery and Mesnil. Undoubtedly they confused here the life-cycles of two very different organisms: whereas if they had proceeded to trace the development further, if their observations had really been made "en détail et sans lacunes," they would soon have discovered their mistake. They give no evidence at all for the conclusion at which they arrived; and unfortunately this observation is quoted by subsequent authors as important evidence in support of the definition of the family Selenidiidae founded by Brasil (1907). I have already discussed this matter in an earlier paper (Ray, 1930). The morphological characters and the behaviour of the two species of *Selenidium* in the gut of *Scolelepis* are quite distinct from those of the organism in question, and, so far as I can discover, these gregarines have no asexual multiplicative phase, whether extracellular or intracellular.

(ii) *Microschizogony.*

In the more posterior portion of the infected gut I have frequently found another type of schizogony. The schizonts here can readily be distinguished from the ordinary schizonts and other forms by a waist-like constriction in the middle of the body, while a large vacuole occupies the whole of the region posterior to the centrally placed nucleus (Pl. XLI, fig. 13). The size of such an individual is about 16μ–$20\mu \times 4\mu$–6μ and it has four to six chromatic dots at its anterior end. With Altmann's stain the vacuole at the posterior end appears to be lined with green granules. The nucleus of this type of schizont divides to give eight (Pl. XLII, fig. 26); and ultimately eight spindle-shaped micromerozoites are formed, which at first have a spiral arrangement (Pl. XLI, fig. 14), but ultimately are placed "en barillet" (Pl. XLII, fig. 27). Each of these merozoites is 8μ–12μ long and 2μ–4μ broad; the nucleus is central in position and shows a loose arrangement of the chromatin, with "blobs" at the periphery. There are two chromatic granules at the anterior end of the spindle-shaped body. These merozoites detach themselves from the group and seem to wander about amongst the epithelial cells, where they may occur singly (Pl. XLII, figs. 34 and 35). At this stage their nuclei become very diffuse.

In their neighbourhood I have often found small protoplasmic masses, 2μ–4μ in diameter, with two to four chromatic granules at one end and six to eight very slender comma-shaped siderophilous bodies, each about 2μ long, projecting from the cytoplasm (Pl. XLII, figs. 31 and 32).

Now it is just in this part of the gut that the female parasites are found, and here also the spores are ultimately developed.

[1] The italics are mine.

Since I have not seen the actual process of fertilisation, I realise that I must not be too confident about the part played by the microschizonts. But my own belief is that they are male parasites which divide into eight motile parts (the micromerozoites), each of which gives rise to a few (six or eight) comma-shaped microgametes. Something analogous is known to occur in Coccidia such as *Caryotropha mesnilii* and *Merocystis kathae*. Very occasionally I have found a group of the minute comma-shaped bodies lying close to a female parasite (Pl. XLII, figs. 32 and 33).

(*b*) The Macrogametocytes.

In the middle part of the gut some of the merozoites formed by the large type of schizont develop into macrogametocytes about 28μ–30μ long by 8μ–10μ broad. A macrogametocyte can be distinguished from an ordinary schizont by the more anterior position of its nucleus, its much paler cytoplasm, and the absence of the chromatic blobs on the membrane of its nucleus. The nucleus is further characterised by the arrangement of its chromatin in a star-like pattern, and its nuclear membrane is almost invisible. There are four to six chromatic dots at the anterior end of the parasite. At this stage the macrogametocytes drop into the lumen of the gut, where they are often found free, and they travel towards the posterior end, where microschizogony is in progress. They there pierce the epithelial cells with their pointed anterior ends and gradually push their way in (Pl. XLI, figs. 10 and 11). Now their posterior ends are drawn out into a short tail, which always stains deeper than the rest of the cytoplasm and is folded back against the body (Pl. XLI, figs. 10 and 12). The nuclear membrane at this stage has practically disappeared, and sometimes three to four strands of chromatin can be seen. This is the stage, I believe, at which the macrogametocytes have attained maturity and are ready to be fertilised.

(*c*) Spore formation.

I have not seen the actual process of fertilisation. Not infrequently I have observed a minute ring-like body lying near a mature female, and occasionally I have seen the same sort of structure lying within its anterior end (Pl. XLI, fig. 12*). This *may* be the modified microgamete: on the other hand, it may be some sort of parasite. If it is the microgamete, then the appearance of the female at fertilisation is very different from what has been observed in other Coccidia. I have never seen any "fertilisation spindle."

That the females are somehow fertilised at this stage is evident, for, without markedly changing in appearance, each divides into two sporoblasts (Pl. XLI, fig. 15), and each of these secretes a sporocyst around it, measuring then about 12μ–16μ in length and 6μ–10μ in breadth. There is no common envelope or öocyst, but the two sporoblasts lie in the same vacuole of the epithelial cell.

The nucleus in each sporoblast then divides. At the beginning of the nuclear division the centrosomes are situated side by side and lie so close to the pellicle

that it is raised in a slight prominence (Pl. XLI, figs. 16 and 17). Later on, as the centrosomes travel away from each other, a spindle is formed between them. The spindle opens out; in my preparations I have seen almost all the stages in the first nuclear division of the sporoblast (Pl. XLI, figs. 17, 18, 19, 20); at the anaphase three rounded chromosomes can sometimes be distinguished at each pole.

Interesting observations have recently been made on the process of reduction division in the Sporozoa. Some of the Coccidiomorpha and certain gregarines have been shown to be haploid organisms throughout the greater part of their life history. The zygote alone is diploid, and reduction takes place at the first division thereof. I hoped to be able to find some corroboratory evidence from my study of *Dorisiella*, but in my preparations the rarity of stages showing the number of chromosomes is disappointing. It would be dangerous to arrive at any definite conclusion from such a meagre material, but what I have seen in the beginning of schizogony and in the division of the sporoblast inclines me to think that *Dorisiella* is also haploid. Three chromosomes appear at each end of the spindle during schizogony, and the number seems again to be three in the sporoblasts. In the young schizont the chromosomes are V-shaped and thin (Pl. XLI, fig. 5) whereas in the sporoblast they are thick and look like short clubs (Pl. XLI, figs. 20 and 21). I have only once seen the first division in the zygote (Pl. XLI, fig. 15) and I could not count the chromosomes in it, so I cannot say exactly at what stage the reduction takes place.

I have little evidence to indicate the fate of the chromatic granules in the later stages of sporozoite formation; but they certainly persist in the zygote, and when the two sporoblasts are formed each gets half the number characteristic of a macrogametocyte (Pl. XLI, figs. 16, 17 and 20). As the sporozoite nuclei are formed, the chromatic dots of the sporoblasts gradually fade and disappear.

By three successive mitotic divisions the nucleus of a sporoblast gives rise to eight nuclei (Pl. XLI, fig. 23), which later on are equally distributed towards either end. The finished spores are smooth-walled, oval in shape, 12μ–18μ long and 6μ–10μ broad (Pl. XLI, fig. 25) and contain eight sickle-shaped sporozoites arranged in a bundle. Each sporozoite is 12μ–14μ long and 2μ–3μ broad; its nucleus is central in position and is provided with a minute karyosome; there is a chromatic dot and a darkly staining area at what I take to be the anterior end of the sporozoite. Ripe spores can be seen escaping from the epithelium and are also found in the lumen of the posterior part of the gut, whence they are carried to the exterior with the excreta. The spores become mixed up with the slimy secretion from the skin, along with particles of sand, eggs, etc., which the worms swallow, thus spreading the infection.

SYSTEMATIC POSITION.

I am convinced that the organism I have described here is something entirely distinct from the gregarines occurring along with it, and I consider

that it is probably an aberrant coccidian. It shows a number of features so unusual that, while feeling fully justified in making a new genus, I am quite unable to say where it should be placed in the present scheme of classification. If my interpretation of what I have seen is correct, then numerous microgametes are developed from one male and at some distance from the female individual; this should place the coccidian somewhere among the Eimeridae. The number of spores and the number of sporozoites these contain are usually held to be features of diagnostic value among these Coccidia. As regards the number of spores, *Dorisiella scolelepidis* agrees with the Cyclosporinae and Isosporinae, but in these sub-families the numbers of contained sporozoites are two and four respectively, whereas in my coccidian there are always eight. Peculiar also is the absence of any definite envelope around the zygote and including the spores. Other unusual features are the invariable occurrence of chromatic granules at the anterior end of the body at all stages, and the migratory habit of the macrogametocytes, which can be seen free in the lumen of the gut and actively penetrating cells further back in the region where spores will be formed. Finally, there is the peculiar microschizogony, which I believe to be associated with the production of microgametes.

I sum up these characters briefly in the following definition of the genus, and must hope that discovery of other allied forms may later enable protozoologists to decide its affinities more exactly.

Dorisiella n. gen.

Chromatic granules present at the anterior end at all stages. Two types of schizogony, with merozoites arranged "en barillet." The macrogametocytes are migratory. No öocyst is developed round the zygote. Two spores are formed, each containing eight sporozoites.

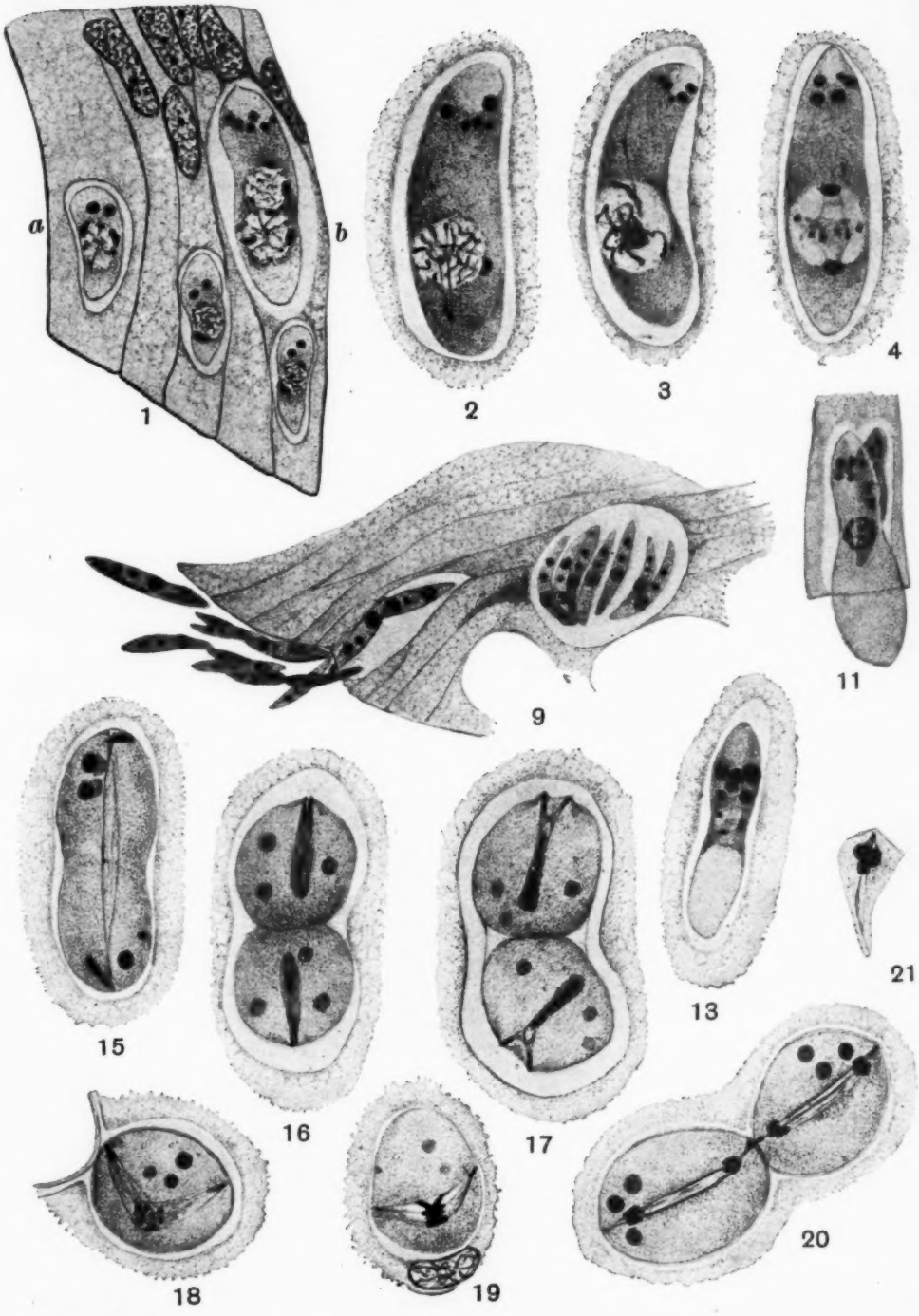
Type-species: *Dorisiella scolelepidis*, in the epithelial cells of the gut of *Scolelepis fuliginosa* Clpde.

I am greatly indebted to Prof. D. L. Mackinnon for all the expert help and criticism which alone have made the work possible. My thanks are also due to Mr Clifford Dobell, F.R.S., for the valuable suggestions he has made from time to time, and to Dr E. J. Allen, F.R.S., and other members of the staff of the Marine Biological Station at Plymouth for the kindness and hospitality which they have shown me during my visits there. I should also like to acknowledge gratefully grants-in-aid received from the High Commissioner for India and from King's College, University of London.

NOTE.

The papers by Harendra Nath Ray, printed in *Parasitology*, vol. **22**, pp. 370–398, 471–478, represent a *Thesis* for the Degree of Doctor of Philosophy in the University of London.

Correction: All measurements given by the author in the present paper are to be reduced by one-quarter—this owing to an error in eye-piece micrometer readings discovered after the proofs were passed.—Ed.



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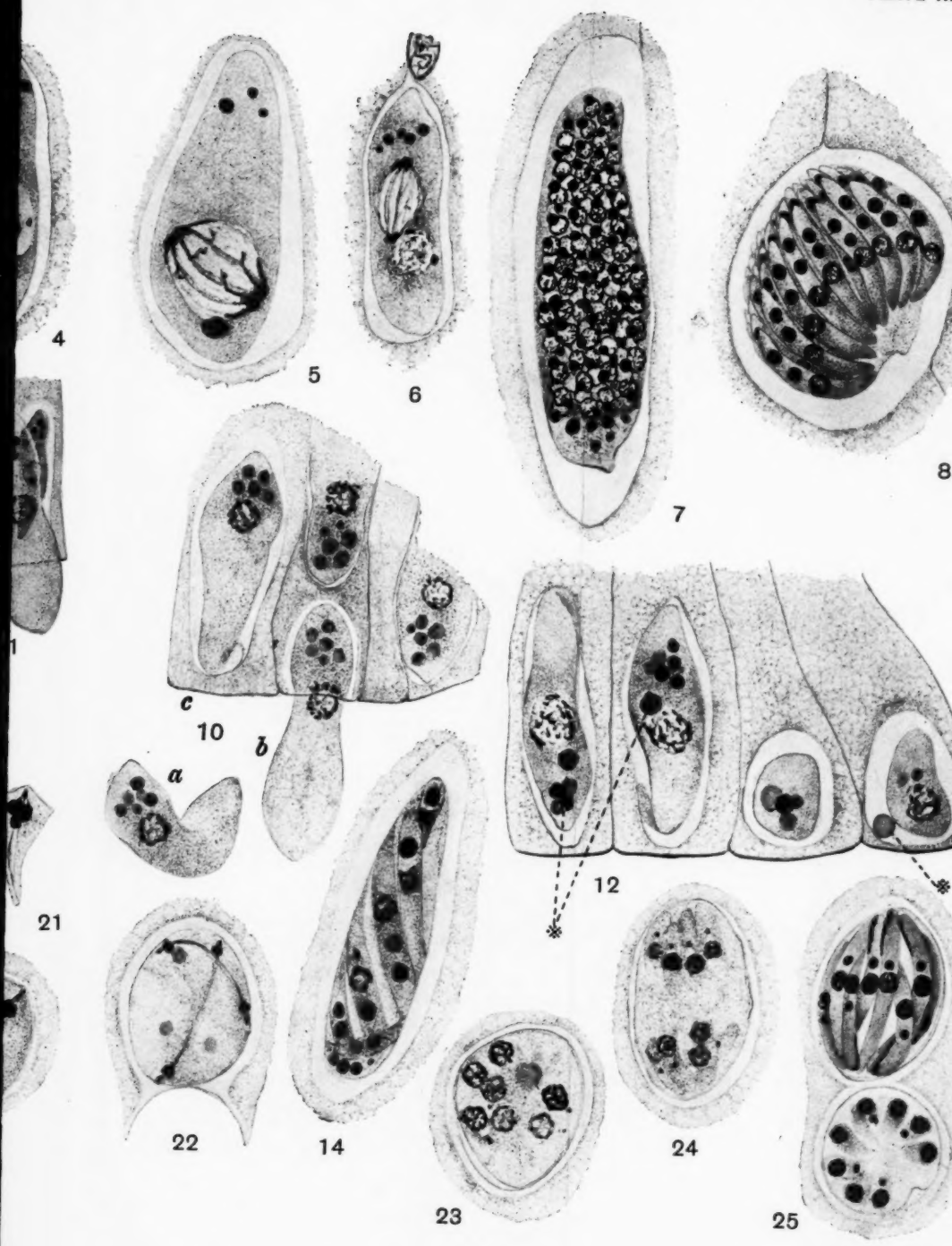
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PLATE XLI

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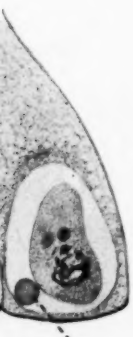

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DESCRIPTION OF PLATES XLI AND XLII.

All drawings were made with the aid of a *camera lucida* and the magnification is ×2100, unless otherwise stated. All figures are of *Dorisiella scolelepidis* and are drawn from sections of the gut of *Scolelepis fuliginosa* fixed with modified Bouin's fluid and stained with Heidenhain's iron haematoxylin. The cilia of the epithelial cells have been omitted in the drawings.

PLATE XLI.

Fig. 1. Schizonts in the epithelial cells of the intestine (*a*) young, (*b*) with two nuclei.

Figs. 2–4. Schizonts with nucleus preparing for division. Fig. 3 shows thread-like chromosomes.

Fig. 5. Schizont. A nuclear spindle showing the V-shaped chromosomes at anaphase. Note the chromatic blob on one side of the spindle. (×4200.)

Fig. 6. Schizont with one spindle and another nucleus preparing for division.

Fig. 7. Schizont with numerous nuclei. Note the old chromatic granules lying towards the clearer end of the schizont.

Fig. 8. Group of macromerozoites.

Fig. 9. Macromerozoites escaping into the lumen of the gut. (×1000.)

Fig. 10. Female parasite (*a*) free in the lumen of the gut and on its way to enter a cell, (*b*) entering an epithelial cell, (*c*) after entering the cell; note the tail-like posterior end at this stage.

Fig. 11. Female parasite entering a cell containing a micromerozoite.

Fig. 12. Mature female gametocytes containing peculiar bodies (* refers to the supposed microgametes in the neighbourhood of their nuclei). Two of the gametocytes are shown in oblique section passing through their anterior ends.

Fig. 13. Microschizont. Note the "waist" and the vacuolar area behind the nucleus.

Fig. 14. Spiral arrangement of the micromerozoites. (×3000.)

Fig. 15. Division of the zygote into two sporoblasts.

Figs. 16, 17. The twin sporoblasts with nuclei preparing to divide. Note how close to each other the centrosomes are placed and the slight elevation of the cytoplasm caused by them.

Figs. 18, 19. Sporoblast. Nuclear division in progress. (Only one sporoblast of a pair is drawn here.)

Fig. 20. Twin sporoblasts showing the nucleus at anaphase. Note the equal distribution of intracytoplasmic chromatic granules and the development of the sporocysts.

Fig. 21. Sporoblast. One end of the spindle at anaphase, with three thick rod-shaped chromosomes. (×4200.)

Fig. 22. Sporoblast with two nuclear spindles. Note the chromatic granules which are fading away. (Only one sporoblast of the pair is drawn here.)

Fig. 23. Spore with six fully formed nuclei and the seventh preparing for division. Note the chromatic granules accompanying the nuclei. The sporocyst is clearly defined.

Fig. 24. Sporoblast, with the beginning of sporozoite differentiation round the nuclei.

Fig. 25. Fully formed spores with eight sporozoites. One spore is shown in transverse section.

PLATE XLII.

All figures are magnified 2500 times unless otherwise stated.

Fig. 26. A microschizont with eight nuclei. Note the chromatic granules at the anterior end and the clear vacuolar area at the posterior.

Fig. 27. A group of micromerozoites.

Fig. 28. The same, cut transversely in the region of the nuclei.

Fig. 29. Two micromerozoites, showing the nuclei with diffuse chromatin. (×1600.)

Fig. 30. A spherical mass of cytoplasm with three chromatic granules and microgametes (?) at its periphery.

Fig. 31. Six microgametes (?) clustered in one vacuole.

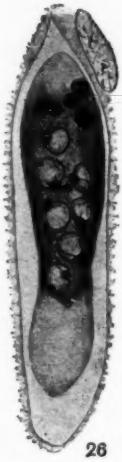
Fig. 32. A portion of the section of the gut wall showing a group of microgametes and one female.

Fig. 33. Three microgametes (?) lying in vacuoles in front of a female.

Fig. 34. A portion of the section of the gut wall showing three mature females and one micromerozoite. (×1050.)

Fig. 35. One micromerozoite lying in the same vacuole with a mature female.

(*MS. received for publication* 3. VI. 1930.—Ed.)



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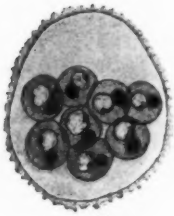

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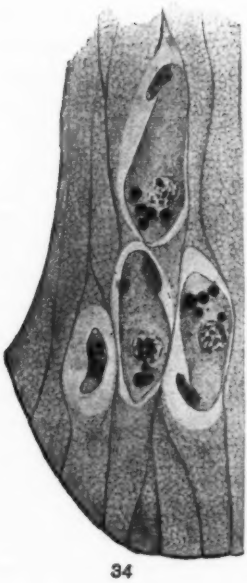

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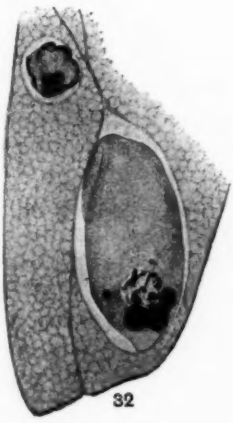

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A CYSTOPHOROUS CERCARIA, *C. PROJECTA* N.SP., FROM THE SNAIL, *HELISOMA ANTROSA*, NORTH AMERICA.

By CHARLES H. WILLEY.

Department of Biology, University College, New York University.

(With 5 Text-figures.)

During the course of a survey of the larval trematodes infecting 950 snails of the species *Helisoma antrosa* collected in the Pocono mountains from a small lake near Henryville, Pennsylvania, more than 10 per cent. were found to be infected with a new cercaria belonging to the group which Ssinitzin (1911) named the cystophorous cercariae. The name *Cercaria projecta* n.sp. is given to the new form.

The snails, collected during the months of October and November, were isolated in separate dishes for from 24 to 48 hours to detect the presence of larval trematodes, and those infected with *C. projecta* were later placed together and kept alive in the laboratory for several months for extended observation. Infections by this form were easily detected through the presence of large numbers of encysted cercariae on the floor of the dish. In the examination of seventy of these heavily infected snails from October 1929 to March 1930, only rediae containing cercariae were found in the digestive gland of the snails. No sporocysts or mother rediae were found.

Redia.

The mature rediae of this species are heavily pigmented, ranging from a yellow colour in the younger stages to a deep orange in the older, larger individuals, and when small and immature they are almost transparent. The rediae are very large, reaching a length of 3·5 mm. and a width of about one-tenth the length. Small, transparent rediae up to 0·40 mm. in length contain no cercariae, and only those larger than 1·3 mm. are found to contain mature, encysted cercariae. They are cylindrical elongated sacs without "feet" or other lateral differentiation of the body wall. The body is broad and blunt at the anterior end (Fig. 1), diminishes in size posteriorly and ends in a narrow point. The pharynx is small, followed by a brownish-coloured sacculate gut, ranging from one-fifth to one-sixth of the body length. A birth pore appears just posterior to the pharynx on the ventral side in the median plane (Fig. 1). When freed from the body of the snail, the rediae move about actively for several

hours by squirming movements produced by the layers of muscles in the body wall. Within the snail, the anterior portion of the mature redia protrudes from the digestive gland and moves back and forth while its posterior end serves to anchor it in the host tissue. Examination of the rediae for parts of the excretory system shows that there are at least sixty flame cells on each side of the body (Fig. 1), distributed generally in groups of three throughout the region posterior to the gut. They are quite small, measuring, while actively beating, from 5 to 8 microns in the length of the flame. From many of these, extremely minute collecting tubules can be traced for short distances but can not be fully worked out due to the heavy orange pigmentation. Running longitudinally in the body wall, a pair of lateral clear vessels of small calibre are visible. These are probably excretory channels, but it is impossible to trace them to any structures which might be identified as excretory pores. The flame cells beat for only a short time (30 minutes to 1 hour) after the rediae are obtained from a freshly killed host, but the action can usually be initiated again by the addition of a small amount of physiological salt solution, run under the coverglass. The large number of flame cells in the redia of *C. projecta* is of interest in its relation to the findings of Wagener (1866), who reported the presence of many flame cells in the sporocyst of *C. cystophora*, in these words: "Ausser vielen gelben, diffusen, von der Leber der Planorbis wohl herrührenden Pigmentflecken unter der Oberhaut zeigt sich auch noch ein sehr entwickeltes Gefässsystem, mit Flimmerlappen reichlich ausgestattet." Investigations of flame cell patterns have not been reported for any stages of the other cystophorous cercariae known. The body cavity of the redia of *C. projecta* is filled with numerous cercariae and germ balls in various stages of development, the number in mature individuals varying from 100 to 200 depending on the size of the redia. From one to seven fully developed cercariae, already encysted (Fig. 1, *ec*), are sometimes to be seen in the anterior half of the body cavity. They are passively pushed back and forth with the developing cercariae, due to contractions of the body of the redia. Ultimately, they are extruded through the birth pore, and the number of encysted cercariae thrown off from a single infested snail in 24 hours reaches as high as 800 or more in heavy infections.

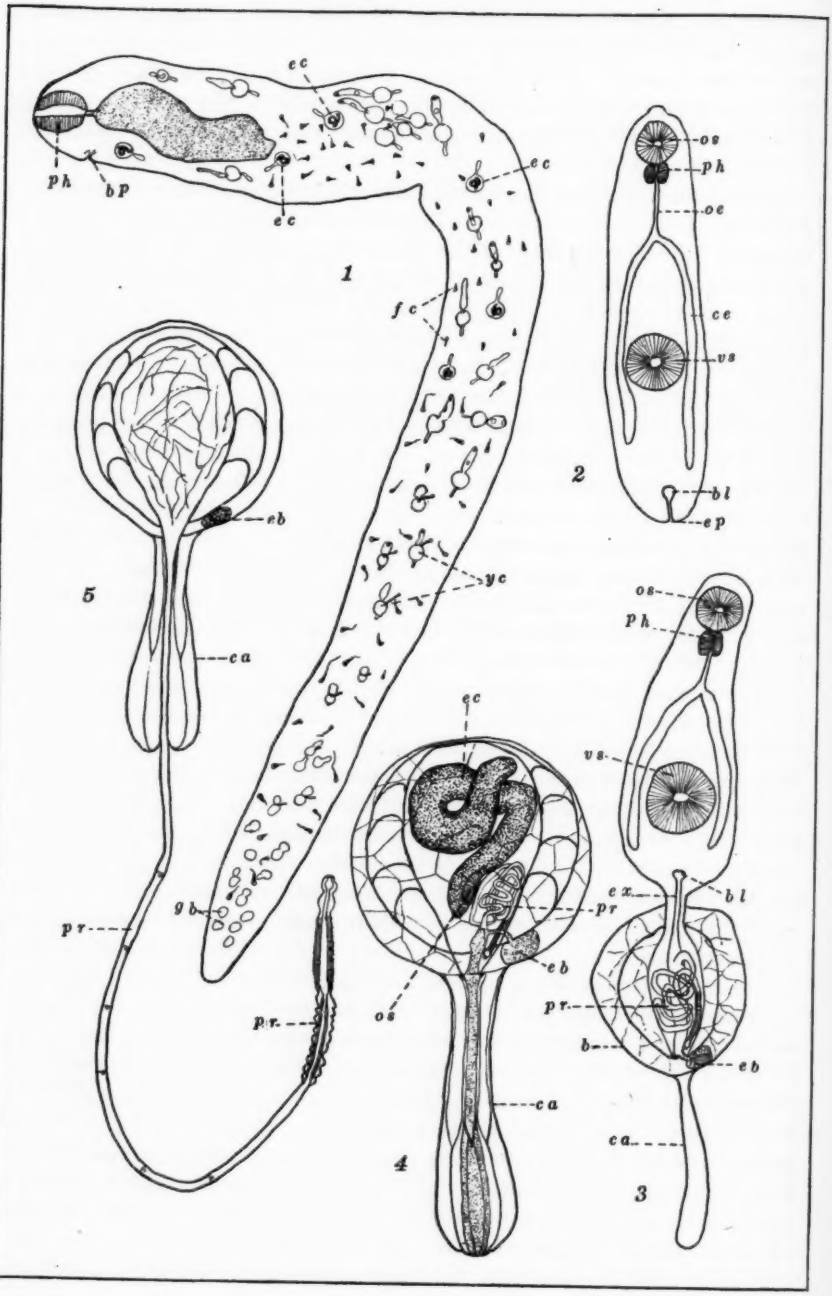
Cercaria.

The body of *C. projecta* is in an undifferentiated condition both before withdrawal into the tail vesicle and after excystment. When mature and freed from the cyst it has a length varying from 0·100 to 0·150 mm. and a width of one-third to one-quarter the length according to the degree of body contraction. The oral sucker is slightly subterminal on the ventral side, and measures 0·020 mm. in diameter. The ventral sucker is slightly larger, and is situated just behind the middle of the body. A prepharynx is lacking, and a small pharynx is followed immediately by the oesophagus which bifurcates in the anterior third of the body to form the intestinal caeca extending back almost to the posterior end of the cercaria. The larva has a rather thick body wall,

without spines, and there is no evidence of a stylet, penetration glands or heavy musculature, indicating that the cercaria is taken into the next host passively rather than by any activity of its own. No genital anlage can be identified. Of the excretory system, only the undivided portion of the bladder and the tube connecting it with the excretory system in the tail can be definitely identified (Fig. 2).

The tail of *C. projecta*, like that of other cystophorous cercariae, is remarkably differentiated, and its development within the redia is similar to that described for other members of the group. Very early in development the posterior third of the larva becomes constricted off and forms a large vesicle which remains united with the body of the developing distome by only a narrow attachment, through which runs the excretory tube (Fig. 3). This vesicular tail, called the bulbus by Faust (1922), is a spherical, double-walled cuticular ball, measuring when the cercaria is mature 0·065 to 0·072 mm. in diameter (Fig. 4). A short, handle-shaped projection arises from the posterior end of the bulbus. This is homologous with the caudal apparatus of *C. invaginata* Faust 1924, and is here designated by the same name. The caudal apparatus of *C. projecta* is provided externally with six longitudinal ridges, giving it a hexagonal appearance in cross-section or in end-view. During the early developmental stages as well as after excystment, a second projection appears from within the tail vesicle. It is in the form of a long, hollow tubule, and is called in *C. californiensis* Cort and Nichols 1920 the excretory projection. During the early stages of *C. projecta* the excretory projection is a short, blunt rod of cells, taking origin from the central column of the tail by a broad attachment. It passes out of the tail vesicle beside the body of the cercaria and extends laterally a short distance. Later, as the larva approaches maturity, an introversion of the excretory projection takes place, and it appears completely coiled up within the bulbus (Figs. 3 and 4). This withdrawal by introversion is apparently the same process as described by Faust (1924) for the lateral excretory tubule of *C. invaginata*. At this time, also, glands within the tail vesicle appear granular and secrete a cyst membrane within the inner cyst wall, and the cercaria begins to withdraw into the cavity formed by this inner cyst membrane. All of these processes occur while the cercaria is still within the redia; the entire cercaria eventually enters the cavity formed by the inner cyst membrane, and the anterior margin of the bulbus is sealed over, completely enclosing the larva within its own tail vesicle. The cercaria squirms about for a short time within its cyst and always takes up a position pointing caudally at the base of the caudal apparatus (Fig. 4). The oral sucker is attached to the base of the coiled-up excretory projection, and the body of the cercaria is always coiled about within the cyst in the same characteristic position, in two loops which are in planes at right angles to each other. In this encysted condition the cercariae pass out of the rediae through the birth pore and later are extruded into the water by the molluscan host.

The excretory system in the tail vesicle is rather a complicated one. From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the undivided portion of the bladder of the cercaria a tube passes backward, and is continuous with a cavity in the central column of the tail (Fig. 3). At the caudal extremity of the tail vesicle a small excretory tube connects the central column with an excretory bulb (Figs. 3, 4), which lies between the inner and outer cyst walls. This bulb contains numerous excretory concretions, and no opening from it to the outside of the cyst could be discerned. From the central column the excretory projection takes origin, and its lumen is continuous with that of the central excretory column. It was impossible to demonstrate with certainty an extension of the excretory system into the caudal apparatus, but this is strongly suspected.

Excystment.

While attempting to obtain cercariae of *C. projecta* free from their cysts for study, a rather remarkable method of excystment was observed. Attempts were made to remove the cercariae from their cysts by means of the digestive ferments, pepsin and trypsin, made up in the usual way in different concentrations, but the larvae did not emerge from the cysts, which were apparently not affected by the enzymes. Some cysts were then placed on a slide in a drop of water and covered with a very thin coverglass (No. 00). While the cysts were under observation, the coverglass was very lightly touched with a needle, with the result that almost all the cercariae emerged from their cysts. On closer examination it was found that all had emerged in exactly the same manner. The excretory projections were all extended at full length and the cercariae were to be found at some distance from the end of the excretory projection, and no breaks could be found in the walls of any of the cyst vesicles. After repeating this method of freeing cercariae several hundred times, using thousands of cysts and obtaining uniform results, the following conclusions were reached. When mechanical pressure is applied on the outside of the cyst, an internal pressure is set up inside the cyst; the excretory projection is forcibly everted caudally through the centre of the caudal apparatus, which seems to split apart. Some internal force then projects the cercaria through the tube of the excretory projection, throwing it a considerable distance from the end

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Fig. 1. Lateral aspect of redia, showing only a few of the many developing cercariae in representative regions.

Fig. 2. Cercaria after excystment.

Fig. 3. Camera lucida drawing of an early stage in the development of a cercaria. The excretory projection has been withdrawn into the bulbus, but the body of the cercaria is still outside of its tail vesicle.

Fig. 4. Camera lucida drawing of an encysted cercaria.

Fig. 5. Empty tail vesicle from which the cercaria has been projected through the lumen of the excretory projection.

b, bulbus; *bl*, bladder; *bp*, birth pore; *ca*, caudal apparatus; *ce*, caecum; *eb*, excretory bulb; *ec*, encysted cercaria; *ep*, excretory pore; *ex*, excretory tube; *fc*, flame cell; *gb*, germ ball; *oe*, oesophagus; *os*, oral sucker; *ph*, pharynx; *pr*, excretory projection; *vs*, ventral sucker; *yc*, cercaria before introversion of excretory projection.

of the projection and from the cyst (Fig. 5). The entire procedure occupies but a fraction of a second, and the observer must watch very intently to see the passage of the larva through the tube. Because of the speed of the reaction, the actual physical explanation of the phenomenon is still a matter of conjecture. The accuracy of the observation has been demonstrated by a very small number of cases in which the action was slower. In these cases, the excretory projection was first fully extended through the caudal apparatus and then the larva passed slowly through the tube, and in a few cases the larva became caught when only a part of the way through. These cases were less than one-half of 1 per cent. of the total number observed, and were not normal. Ordinarily, the process is very rapid.

The passage of the cercaria through the tube is absolutely passive, since it possesses no organs of locomotion which could propel it. During the passage it becomes very much elongated and narrow in cross-section, but beginning almost immediately after being shot from the mouth of the excretory projection, it slowly becomes shorter and thicker to attain the proportions shown in Fig. 2, and moves about very slowly for an hour or two with the aid of its suckers. During this period a terminal excretory pore is very prominent, having just severed its connection with the tail structures.

The excretory projection of *C. projecta*, when thus everted from the cyst through the side opposite to that through which it was drawn in, shows considerable differentiation (Fig. 5). Its length is equal to about six times the diameter of the cyst. It is a hollow tube, apparently with cuticular walls, and is provided with several transverse joints which make it flexible. The terminal fifth of the tube shows a further differentiation which was visible, pointing caudally, even when the structure was coiled up within the tail vesicle. In the first half of this region the cuticular walls are irregular externally and thickened, producing a very narrow lumen (Fig. 5). This is followed by a portion with wider lumen and walls which appear beaded. From here a wide bulbular structure leads to the outside through a terminal pore. The association of the excretory projection with the excretory system in *C. projecta* is certain, and it seems that the larva utilises the complicated structures of this system in the tail as a means of excystment.

Concerning the rôle that this type of excystment plays in the life history of *C. projecta*, little more than speculation is at present possible. It is certain that the cyst enters the next host passively. Experiments show that the cercariae were not released from their cysts by digestive ferments and that mechanical means are required for the release. In *C. cystophora* Wagener 1866, the only cystophorous cercaria for which the life history is known (Ssinitzin, 1905), the next host is the dragon-fly larva, *Colopteryx virgo*. In such an insect host or in a small crustacean, the action of the masticatory apparatus would provide the necessary mechanical pressure for excystment of *C. projecta*, and the cercaria would be forcibly thrown a considerable distance from the cyst, perhaps into some protecting folds or crevices in the mouth or gullet of the

host; and, while its empty cyst might be regurgitated, the larva could with safety gradually make its way into the appropriate location in the secondary host. Experiments showed that the cercariae of *C. projecta*, freed after being for 6 weeks in the encysted state in the laboratory, were just as active as those released after only a few days or hours of encystment, but if left much longer the cercariae either died within their cysts, or the process of excystment was abnormal, often bursting the cyst walls at various places and showing little or no activity after being released.

DISCUSSION.

C. projecta is a member of a group known as the cystophorous cercariae, characterised by possession of a vesicular tail with various appendages upon it. Eleven other cercariae of this type have been described, and of these the life history of only one is known. The first species recorded is *C. cystophora*, described by Wagener (1866) from *Planorbis marginatus*, and the life history of this form was reported by Ssinitzin (1905). Four species are marine. Of these, Pelseneer (1906) described *C. appendiculata* from *Natica alderi* and *C. vaullegeardi* from *Trochus cinerarius*; and Ssinitzin (1911) described *C. saggitarius* from *Cerithiolum exille* and *C. laqueator* from *Rissoa venusta*. Among the freshwater forms, *C. capsularis* from *Cleopatra bulimoides* was described by Sonsino (1892) and later Looss (1896) supplemented this description with a report on the developmental stages, definitely establishing it as one of the cystophorous group. Since that time, the following species have been reported: *C. yoshidae* Yoshida 1917 from *Melania libertina* in Japan, *C. californiensis* Cort and Nichols 1920 from *Physa occidentalis* in California, *C. macrura* Faust 1921 from *Planorbis pfefferi* in South Africa, *C. syringicauda* Faust 1922 from *Melania ebenina* in China, and *C. invaginata* Faust 1924 from *Melania cancellata* in China. *C. projecta*, described in the present paper, is the second species to be reported from America. In these few forms, all of which have many major characteristics in common, there is considerable confusion concerning homologies, and different names are applied to homologous structures in different forms.

Although the life history is known for only a single species, suggestions have been made concerning further hosts of other forms. Ssinitzin (1911) reported that *C. saggitarius* encysts within the redia, and since he failed to find any cysts outside of the rediae, he assumes that the snail is eaten entire by the next host, a bird or a fish. Cort and Nichols (1920) suggest that *C. californiensis* is transferred to its next host through the eating by a vertebrate of the entire infected snail. It appears that only crushed snails were used in the investigations, and this fact may explain the failure to find encysted stages. As a matter of fact, crushed specimens of *Helisoma antrosa*, infected with *C. projecta*, very often yield no encysted stages outside of the rediae, and in many cases no encysted cercariae are visible even within the rediae. If only a few infections were studied, and if these were all from crushed snails, it

might readily be concluded that no encystment occurred. Because of insufficient material, and due to the method of study employed, Faust (1922) reports that in *C. syringicauda* no encystment of the body itself seems possible, but there may be a stage later than those observed when encystment within the bulbus does occur, and for *C. invaginata* Faust (1924) reports that encystment was not observed, but probably takes place later. Since the same fundamental plan is found in all the different forms with respect to the tail vesicle and its appendages, it seems quite likely that at least some of those forms for which no encystment has been described do encyst. It is probable also, as suggested by Ssinitzin (1911), that the life cycles of the members of this group will be similar to that of *C. cystophora*, worked out by him (Ssinitzin, 1905). In this form the cyst passes into a dragon-fly larva, *Colopteryx virgo*, as its second intermediate host, and later develops into *Halipegus ovicaudatus* found in the mouth cavity of the European frog. A similar life cycle is suspected for *C. projecta*.

SUMMARY.

A new species of larval trematode, *C. projecta*, infects *Helisoma antrosa*. The very small distome cercaria is ontogenetically young, showing no traces of reproductive anlagen even after excystment. It belongs to the cystophorous cercariae, and has a vesicular tail into which the body of the cercaria withdraws when mature. In this encysted state the cercaria is expelled from the snail and awaits ingestion by the next host. The redia is a large, elongate sac, without "feet," possessing a small pharynx, short intestine and an excretory system consisting of a longitudinal channel and at least sixty flame cells on each side of the body. Excystment is caused by mechanical pressure which releases a force within the cyst, which projects the cercaria very rapidly through a long cuticular tube, the excretory projection, to a considerable distance away from the cyst. Various complex appendages of the tail vesicle along with parts of the excretory system play a large part in this process of excystment. As postulated, the purpose of this type of excystment is to throw the larva away from its cyst, to prevent it from being regurgitated from the body with the empty cyst.

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ON THE OCCURRENCE IN MAN OF STRAINS OF *T. GAMBIENSE* NON-TRANSMISSIBLE CYCLICALLY BY *G. PALPALIS.*

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From the Human Trypanosomiasis Institute, Entebbe, Uganda.

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I. Introduction.

In the *Final Report of the League of Nations International Commission on Human Trypanosomiasis*, published in 1928, an account was given of experiments on the transmissibility of *T. gambiense* by *G. palpalis*. These experiments, though admittedly incomplete, suggested certain conclusions, the chief of these being that prolonged sojourn of *T. gambiense* in an individual, whether man or animal, in which the trypanosome produces a slowly progressive illness, tends to reduce and eventually to abolish the transmissibility of the strain by tsetse.

In the series of 19 human strains of *T. gambiense* examined by the Commission several were pronounced to be non-transmissible when first isolated from man. In his review of the *Report* published in the *Tropical Diseases Bulletin*, **25**, No. 10, Prof. Yorke rightly observes that the vital deduction reached from the investigation of these 19 strains is that 4 of the 12 studied in the human patient, *i.e.* 33·3 per cent., were non-transmissible cyclically by laboratory bred *G. palpalis*. After remarking on our ignorance why, even in the most favourable conditions, only a comparatively small percentage of tsetse ever becomes infected with trypanosomes, he exposes the admitted weakness of my case, namely the small numbers of flies employed in the examination.

He assembles in a table the data given in Part I of my report, and it is column 4 of this table, headed "Total flies dissected," which supplies the force of the indictment.

The figures quoted in Prof. Yorke's table relate only to the experiments of Part I, namely those performed either on the patient himself or on the animal

in which the strain first reached the laboratory. In these preliminary examinations, 218 was the greatest number of flies employed for any one strain, and many of the totals were considerably less.

Now long experience of this kind of work has taught me that even with *T. rhodesiense*, which is as a general rule more readily transmissible by *G. palpalis* than is *T. gambiense*, it is rare to find more than 10 per cent. of the flies infected with trypanosomes. Higher percentages are sometimes obtained, but as a rule only in experiments where less than 200 flies have been used.

Figures such as those selected by the reviewer certainly do not warrant far-reaching conclusions. But, as a matter of fact, they represent only a part of the evidence put forward. The data given in Part II of the paper are not included in Prof. Yorke's table; yet in the case of several of the strains they strengthen and confirm the conclusions foreshadowed in Part I. This is particularly true for the alleged non-transmissible strains, which after all are the most important. Let us take three of these, Nos. III, VI and VIII. These three strains when first isolated from man, *i.e.* as set forth in Part I, were exposed to 127, 150 and 183 flies respectively. In Part II the behaviour of these strains in subsequent animal passages is described, and we see that a further 547, 435 and 204 flies respectively were employed, so that the total available evidence that these strains were indeed non-transmissible by tsetse, presented in the manner adopted by the reviewer, was as follows:

Strain	Date	Total flies dissected	Total infected flies	% of infected flies	Number of infected flies alive at 25th day: With glands infected	Number of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index[1]
III	May–Oct. 1926	674	57	8·4	0	53	0	0
VI	May–July 1926	585	11	1·8	0	9	0	0
VIII	June–Oct. 1926	387	12	3·1	0	8	0	0

And, in considering this table, it is important to note the high proportion of the infected flies that lived 25 days, *i.e.* long enough for the appearance of metacyclic forms in the glands if the strain were still capable of producing them.

Strains XVII, XVIII and XIX of the reviewer's table were not subjected to any subsequent examination. I had originally intended to omit these from the report, but on the advice of my colleagues I eventually decided to include them. In the light of subsequent experience the number of flies used in the examination of these last three strains is certainly inadequate, particularly in the case of Strain XVII, where one fly showed a gut infection.

In addition to the human strains dealt with by the reviewer, the Commission's *Report* contains the account of another strain, recovered from a situtunga antelope killed on Damba Island, adjoining the Sesse Group in Lake Victoria, which was also subjected to thorough examination and found to be

[1] For an explanation of the Transmissibility Index, cf. *Parasitology*, **20**, p. 427.

non-transmissible. As this antelope lived in a locality heavily infested with *G. palpalis*, where, moreover, the fly fed freely on the game, this strain—No. XXIII of the *Report* series—is worthy of mention here. This trypanosome, known to us at Entebbe as the Damba trypanosome, in its reactions resembles a mild *T. brucei*, and this fact adds greatly to its importance to the present discussion.

A total of 958 clean *G. palpalis* were fed upon animals infected with Strain XXIII; all these flies were dissected and only two found to contain flagellates, the infection in each being exceedingly scanty and consisting of only a few scattered trypanosomes in the whole gut contents.

When the Commission disbanded, my studies were not nearly completed, but circumstances made publication inevitable. The session was originally fixed at 12 months, but as the year went by it became obvious that the time was hopelessly insufficient to ensure any definite results in any of our undertakings. Another 6 months were granted, and at the end of that time it was necessary to assemble the experimental data and see where they pointed. By then certain conclusions were taking shape and they were formulated in the paper under review.

The experience of the 2½ years that have elapsed since the writing of the *Final Report* has gone far to confirm some of its tentative conclusions; others it is true will need qualification, but it was made clear that the work was to be continued.

On the main question, the existence of non-transmissible human strains in nature, there is fortunately nothing to recant, and we can now turn to the new evidence.

In the present paper are collected all the experimental data, accumulated from June 1927 up to the date of writing, on the subject of non-transmissible human strains of *T. gambiense*.

We are not concerned here with strains that were transmissible when first isolated and have lost this character during their maintenance at the laboratory. Evidence is still being collected on that subject, which will receive separate treatment at a later date.

First of all we will take the experiments performed with a strain of *T. gambiense*, recovered from a European in a fairly advanced stage of trypanosomiasis. The transmissibility of this strain by *G. palpalis* has, I submit, been exhaustively investigated, and affords an excellent example of the phenomenon we are considering.

Discussion of the ways and means by which loss of transmissibility takes place will be resumed on another occasion, when the studies now in progress at Entebbe have cleared up certain points that are still obscure.

In his review alluded to above, Prof. Yorke expresses doubt about the desirability of conveying through the medium of the *Tropical Diseases Bulletin* anything of the nature of criticism. I feel sure that those whose lot it is to work in isolated laboratories in the Tropics, and who are denied both the healthy

stimulus of discussion with their own kind and the ample literature available at a well-equipped Research Institute at home, will welcome such criticism in the spirit in which it is offered.

I trust that the reviewer will see in the present paper the realisation, in my own case, of the hopes expressed in his introductory remarks.

To make clear the difficulties attending an investigation of this kind, it should be explained that each "experiment" consists of a box containing originally 50 laboratory bred tsetse. Some of these die during the manipulations of the first few days, either before they have fed at all or so soon after their infected feed that it is impossible to decide whether any flagellates they may contain are merely passively surviving or are really established in the fly's gut; these are rejected. Each box has then to be attended to for about 30 days, before the final dissection reveals its secrets. It often happens that a considerable proportion, sometimes all, of the infected flies in an experiment die before the 25th day, and in consequence nothing is learnt about the power of the strain to invade the glands. Added to these difficulties there is the variability of the trypanosome itself from day to day in its infectivity to fly. Although nothing new has been learnt about this point and it is still uncertain to what extent, if at all, individual flies vary in their liability to infection, there is no doubt in my mind that the infectivity of a strain of trypanosomes to tsetse does vary from day to day. These variable factors combine to introduce an exasperating uncertainty into these examinations, which has gradually led to the elaboration of the present technique. And lastly, the tax upon the monkey is so great that it is rarely justifiable to apply more than two tests to the same animal: this renders difficult the study of the changes undergone by a strain in any individual host.

A "test" consists of a collection of experiments applied to an infected animal over a period usually of 2 or 3 weeks, each box being fed twice on the infected animal, on alternate days with a day's starvation in between.

With the European strain the details of the experiments are summarised, but in the case of the other three strains they are given in full.

The object of this paper is to secure the recognition of what I believe to be a biological principle: hence the large number of flies employed in the examination of the European strain. For practical purposes, however, in a comparative study of the transmissibility of a number of different strains, a much less elaborate test will suffice, provided that if there are any infected flies, a sufficient number of them lives 25 days.

The spread of the human trypanosomes by tsetse in nature depends on their ability to establish themselves in the glands of the fly. After this power has been lost a strain remains for a time infective to the intestine of the fly, and finally this property in its turn disappears. A test in which certain flies are found to carry a heavy intestinal infection, none, or only a small proportion of these infected individuals living as long as 25 days, is consequently null and void: the strain may or may not be transmissible.

II. Experiments with the European strain.

(*a*) *Account of the principal events leading up to the isolation of the strain dealt with in this paper.*

At the beginning of 1925 the European from whom this strain was subsequently isolated was a strong healthy man of about 33 years of age, of robust physique and constitution. Early in that year he was sent to Arua in the West Nile district of Uganda, part of which is an area officially declared infected with *T. gambiense*. In this district he remained for about 2 years, suffering repeatedly from fever, and on several occasions malaria parasites were found in his blood. As time went on these febrile attacks became more and more frequent and difficult to control. He returned to headquarters early in 1927 and malaria and microfilariae were found in his blood, but no trypanosomes. At this time he showed no signs of lymphadenitis.

In March 1927 he was invalided home to England. Here microfilariae were again found in his blood. After treatment and rest he returned later in the year to Uganda, improved in health. Soon after his return he began again to suffer from attacks of fever, and his health steadily deteriorated. His case baffled all attempts at diagnosis. Trypanosomiasis was considered on several occasions, but no trypanosomes were found and there was no lymphadenitis. Finally, in January 1929, a series of stained thick blood-films taken daily revealed the presence of trypanosomes, and an examination of the cerebrospinal fluid showed trypanosomes and a cell count of 44 per cubic mm.

From the middle of 1928 there had been signs, recognisable by his friends, of depressed vitality, and in September, when he went away on local sick-leave, somnolence was a definite symptom. The only arsenical drugs ever administered were Stovarsol, of which a course was given by the mouth early in 1928, and "N.A.B.," two injections of which were given in October of the same year.

The history of the case is especially interesting when we endeavour to decide when the original infection occurred. The condition of the cerebrospinal fluid in January 1929 suggests that the disease was then of long standing. On his return to Uganda from leave in October 1927 he was not in full health in spite of extensive quinine treatment and the benefits of complete change of scene and climate.

There is, however, another possible explanation. Around Port Bell, 7 miles from Kampala township, there have been in recent years occasional cases of human trypanosomiasis in natives, some of which were in all probability infected locally. These cases have always been traced either directly to the Eastern Province lake-shore or to the intervention of an infected native from that region who had settled near Port Bell. It is, therefore, conceivable that infection took place near Kampala, and this would date the onset to the end of 1927 or early 1928.

By September 1928 increased somnolence was a definite symptom, so that

if this second explanation is adopted we must assume a rapid and acute illness developing under the eyes of medical officers who were all more or less familiar with the symptoms of human trypanosomiasis. This, to say the least, is improbable. A chronic infection of an unusual character and complicated by malaria and filariasis might, on the other hand, easily elude diagnosis for some time, and I feel that the history of the case definitely admits the possibility that the original infection took place in the West Nile infected area early in 1927. This, too, is the belief held by the patient himself.

The point that concerns us here is the length of time this strain had been present in the patient before it came under examination at the laboratory. According to which alternative explanation is adopted we see that the infection had lasted either 2 years or approximately 12 months, and was untreated save for a course of Stovarsol and two injections of "N.A.B." given in October 1928. When subjected early in 1929 to a full course of tryparsamide the patient began immediately to improve, and at the time of writing, a year after the cessation of treatment, he shows no signs of the disease.

(*b*) *Experiments performed with the European strain.*

Table I gives the history of the strain from its first isolation in Monkey 507 on 18. i. 29 by subcutaneous inoculation of 10 c.c. of the patient's citrated blood. The three dates accompanying each monkey's experimental number in this table relate respectively to the inoculation of the infected blood, the first detection of trypanosomes in the peripheral blood and the death of the animal. These dates should be compared with those given in Table II.

In Table I only animals subjected to fly tests are mentioned.

Table I.

507	18. i. 29				
	29 i. 29				
	not recorded; about end of August 1929				
506	8. iii. 29				
	18. iii. 29				
	30. ix. 29				
547	26. iv. 29	606	30. ix. 29	607	30. ix. 29
	7. v. 29		5. x. 29		8. x. 29
	13. ix. 29		25. xi. 29		13. ii. 30
601	10. ix. 29				
	23. ix. 29				
	16. i. 30				

Table II shows summaries of the fly experiments performed upon this strain, and its infectivity to *G. palpalis* in different animal passages. In the "Date" column is shown the period during which the flies were applied to the infected animal.

The following are the points of interest in Table II:

(1) Wherever more than one test was applied to a monkey the percentage of infected flies diminished in each successive test.

(2) Of a total of 66 infected flies, 50, *i.e.* 75 per cent., lived 25 days or more: this high percentage of survivors greatly enhances the value of the investigation.

Table II.

Animal	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Trans-missibility index	Total
M 507	3–19. ii. 29	226	8	3·5	0	7	0	0	1543
	5–24. iii. 29	385	9	2·3	0	7	0	0	
	19. v.–11. vi. 29	483	9	1·8	0	6	0	0	
	22. vi.–11. viii. 29	449	3	0·6	0	2	0	0	
M 506	23. iii.–18. iv. 29	343	4	1·1	0	3	0	0	858
	19. v.–11. vi. 29	515	5	0·9	0	5	0	0	
M 606	14. x.–14. xi. 29	456	6	1·3	0	4	0	0	—
M 547	22. v.–21. vi. 29	430	10	2·3	0	8	0	0	—
M 601	24. ix.–14. x. 29	425	6	1·4	0	3	0	0	—
M 607	15. x.–11. xi. 29	375	6	1·6	0	5	0	0	900
	19–31. xii. 29	525	0	0	0	0	0	0	

(3) The percentage of infected flies in the *first* tests on each animal is fairly constant. Of the 2255 flies employed in all these first tests 40, *i.e.* 1·7 per cent., were infected; and of these 40 flies, 30, *i.e.* 75 per cent., lived 25 days or longer.

In the course of these experiments 4612 flies were fed upon animals infected with this European strain; 66 flies developed massive gut infections, but in none were the salivary glands infected; 75 per cent. of the infected flies lived 25 days or longer.

III. Some new non-transmissible strains of *T. gambiense* not hitherto described.

(*a*) *Strain Odira.*

From a native of the West Nile District of Uganda who was admitted to hospital at Kampala one month before he died of trypanosomiasis. When first seen was obviously in an advanced stage. Trypanosomes were found in glands and blood. No history was obtainable. During his brief stay in hospital he suffered from irregular pyrexia that never reached 101° F. He was drowsy and irritable and abnormal mentally.

On 6. x. 28 Monkey 479 was inoculated with citrated blood and with gland juice from this patient. On 16. x. 28 the patient died in hospital. On 31. x. 28 trypanosomes were first found in the monkey's blood, stained thick films of which had been examined daily. On 13. xii. 29 the monkey was killed by a jackal; it was then thin and feeble but not somnolent.

Table III.

Exp. no.	Infected animal	Day of experiment on which dissection began	No. of flies alive on 25th day	No. dissected during the experiment: Males	Females	Total	Duration of experiment in days	No. of flies containing flagellates: In gut only	In gut and salivary glands	Ages in days of the infected flies dissected during experiment	Dates of infecting feeds
116	M 479	2	24	33	22	55	29	1	0	,13,	7, 8, 10. xi. 28
117	,,	3	28	31	13	44	30	0	0	—	7, 9, 11. xi. 28
118	,,	3	21	26	20	46	28	0	0	—	14, 16, 18. xi. 28
119	,,	3	23	24	17	41	30	0	0	—	15, 17, 19. xi. 28
120	,,	3	25	34	33	67	28	0	0	—	20, 22, 24. xi. 28
121	,,	4	37	28	38	66	29	0	0	—	20, 21, 23. xi. 28
122	,,	6	24	24	13	37	32	0	0	—	26, 28, 30. xi. 28
123	,,	2	37	30	35	65	31	0	0	—	27, 29, 30. xi. 28
128	,,	4	26	17	23	40	31	1	0	,5,	1, 2, 4. xii. 28
129	,,	4	20	17	18	35	34	0	0	—	1, 3. xii. 28
187	,,	6	34	29	25	54	30	0	0	—	2, 4. ii. 29
189	,,	5	14	19	22	41	29	0	0	—	3, 5. ii. 29
193	,,	4	18	20	15	35	29	0	0	—	4, 6. ii. 29
195	,,	9	24	23	9	32	30	0	1	,30,	5, 7. ii. 29
204	,,	6	18	14	22	36	31	0	0	—	8, 10. ii. 29
207	,,	6	5	11	25	36	30	1	0	,6,	11, 12, 14. ii. 29
216	,,	6	13	19	18	37	30	0	0	—	13, 14, 16. ii. 29

Note. In the penultimate column of these tables a comma indicates that the figure following it relates to a gut-only infection; a semicolon indicates a gland infection.

Summary.

Animal	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	Total	% of gland infections	Transmissibility index
M 479	Nov. 1928	496	2	0·2	0	0	0	*0*
,,	Feb. 1929	271	2	0·7	0	1	0	*0*

(*b*) *Strain Pio.*

Derived from a lunatic patient admitted to hospital shortly before his death. Presumably infected in the West Nile area, but no accurate history available. Patient very emaciated.

Monkey 554 was inoculated with citrated blood from this patient on 29. v. 29, 18 days before his death.

Experimental investigation of this strain with laboratory bred *G. palpalis* gave the following results.

Table IV.

Exp. no.	Infected animal	Day of experiment on which dissection began	No. of flies alive on 25th day	No. dissected during the experiment: Males	Females	Total	Duration of experiment in days	No. of flies containing flagellates: In gut only	In gut and salivary glands	Ages in days of the infected flies dissected during experiment	Dates of infecting feeds
401	M 554	6	34	23	24	47	31	0	0	—	6, 8. vii. 29
402	,,	5	38	23	20	43	31	0	0	—	7, 9. vii. 29
409	,,	22	32	27	17	44	28	0	0	—	12, 14. vii. 29
418	,,	6	32	18	30	48	32	0	0	—	19, 21. vii. 29
420	,,	6	23	20	19	39	31	1	0	,31,	20, 22. vii. 29
422	,,	10	34	22	22	44	29	0	0	—	22, 24. vii. 29
425	,,	8	51	22	32	54	29	2	0	,29,29,	23, 25. vii. 29
431	,,	5	28	21	16	37	28	0	0	—	26, 28. vii. 29
433	,,	7	40	24	28	52	30	0	0	—	27, 29. vii. 29
436	,,	7	26	24	24	48	28	0	0	—	29, 31. vii. 29

Summary.

Animal	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
M 544	July 1929	456	3	0·6	0	3	0	*0*

Note. It will be noted that all the infected flies lived 25 days or longer.

(*c*) *Strain Eli.*

The patient was a small boy suffering from trypanosomiasis sent to Entebbe on account of his failure to respond to treatment with a full course of tryparsamide. He came from the West Nile district. On treatment with Bayer 205 trypanosomes immediately disappeared from his blood and glands.

Monkey 641 was inoculated with this boy's blood on 6. xii. 29: trypanosomes were first seen on 27. xii. 29. The monkey died on 28. iv. 30.

Fly experiments with this monkey gave the following results.

Table V.

Exp. no.	Infected animal	Day of experiment on which dissection began	No. of flies alive on 25th day	No. dissected during the experiment: Males	Females	Total	Duration of experiment in days	No. of flies containing flagellates: In gut only	In gut and salivary glands	Ages in days of the infected flies dissected during experiment	Dates of infecting feeds
725	M 641	7	30	22	19	41	30	0	0	—	28, 30. xii. 29
726	,,	17	31	21	16	37	29	0	0	—	29, 31. xii. 29
728	,,	7	24	19	13	32	30	0	0	—	29, 31. xii. 29
731	,,	10	36	15	23	38	29	0	0	—	1, 2. i. 30
732	,,	6	35	13	27	40	29	0	0	—	1, 3. i. 30
741	,,	10	43	26	28	54	30	0	0	—	4, 6. i. 30
744	,,	5	34	20	21	41	29	0	0	—	5, 7. i. 30
748	,,	7	13	8	11	19	29	0	0	—	7, 8, 10. i. 30
752	,,	16	31	17	20	37	29	0	0	—	9, 11. i. 30
754	,,	7	43	27	27	54	31	0	0	—	10, 12. i. 30
758	,,	8	29	16	24	40	31	0	0	—	13, 15. i. 30
760	,,	14	37	15	24	39	30	0	0	—	14, 16. i. 30
773	,,	5	32	21	19	40	28	0	0	—	22, 24. ii. 30
776	,,	6	30	17	18	35	29	0	0	—	26, 27. ii. 30
780	,,	11	34	15	22	37	28	0	0	—	28. ii. & 2. iii. 30
785	,,	9	45	23	31	54	31	0	0	—	4, 6. iii. 30
788	,,	10	34	10	39	49	31	0	0	—	5, 7. iii. 30
794	,,	8	45	24	30	54	29	0	0	—	9, 11. iii. 30
800	,,	7	30	25	25	49	28	0	0	—	12, 14. iii. 30
803	,,	6	39	38	22	60	28	0	0	—	13, 15. iii. 30
805	,,	6	33	22	23	45	31	1	0	,9,	14, 16. iii. 30
809	,,	8	30	23	24	47	29	0	0	—	16, 18. iii. 30
811	,,	3	41	37	25	62	28	0	0	—	17, 19. iii. 30
820	,,	6	38	22	24	46	29	0	0	—	24, 26. iii. 30

Summary.

Animal	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
M 641	Dec. 1929–Jan. 1930	472	0	0	0	0	0	*0*
,,	Feb. 1930–Mar. 1930	578	1	0·1	0	0	0	*0*

All these three strains were *T. gambiense*. Strains Odira and Pio were both derived from cases in the last stages of their infection, shortly before the patient's death. The boy Eli, on the other hand, was not apparently very ill,

in spite of his course of arsenic and the relapse or complete resistance of his infection. At the time of his visit to Entebbe he was also suffering from malaria, schizonts and crescents being both present in his blood. He improved wonderfully the moment Bayer 205 was administered. The duration of Eli's infection is uncertain, but it is certain that he had been infected at least 6 months before his strain was isolated in Monkey 641.

According to my view, it is the prolonged struggle between the mammal and the trypanosome that results in the loss by the parasite of transmissibility by tsetse. A chronic but steadily progressive disease is, I think, most likely to produce this result. But as already noted in the *Final Report* of the Commission, in the final stages of a long-standing infection in man, as judged by the alteration of the cerebro-spinal fluid, a strain does not always lose its transmissibility. Quite recently a strain was isolated from a patient on his death-bed, a man brought in shortly before his death by friends from whom no reliable history could be elicited. The results of examination of this strain were as follows.

Monkeys 684 and 685 were inoculated from the patient on 18. ii. 30, the day before his death. Monkey 684 first showed trypanosomes on 25. ii. 30; 685 on 10. iii. 30.

Table VI.

Fly experiments.

Exp. no.	Infected animal	Day of experiment on which dissection began	No. of flies alive on 25th day	No. dissected during the experiment: Males	Females	Total	Duration of experiment in days	No. of flies containing flagellates: In gut only	In gut and salivary glands	Ages in days of the infected flies dissected during experiment	Dates of infecting feeds
791	M 684	5	43	21	39	60	29	0	0	—	7, 9. iii. 30
793	,,	6	36	27	33	60	30	0	0	—	8, 10. iii. 20
797	,,	5	32	23	23	46	28	4	0	,11,12,28, 28,	11, 13. iii. 30
799	,,	10	53	29	27	56	31	0	2	;27;30;	12, 14. iii. 30
807	,,	7	32	16	24	40	31	0	0	—	15, 17. iii. 30
810	,,	6	38	22	23	45	32	1	0	,32,	16, 18. iii. 30
814	,,	4	46	26	38	64	32	0	1	;32;	18, 20. iii. 30
816	,,	7	35	32	16	48	30	1	2	30;30;30;	20, 22. iii. 30
819	,,	7	32	23	18	41	30	1	1	30;30;	23, 25. iii. 30
801	M 685	7	35	11	33	44	31	0	1	;31;	12, 14. iii. 30
802	,,	5	32	14	24	38	30	0	0	—	13, 15. iii. 30
806	,,	5	30	33	20	53	29	0	0	—	14, 16. iii. 30
808	,,	6	28	23	26	49	31	0	2	;31;31;	15, 17. iii. 30
813	,,	6	40	21	29	50	30	0	1	,30,	18, 20. iii. 30
815	,,	5	36	25	25	50	31	0	0	—	19, 21. iii. 30
817	,,	6	51	34	21	55	31	0	0	—	21, 23. iii. 30
818	,,	6	38	28	28	56	31	0	0	—	22, 24. iii 30

Summary.

Animal	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	Total	% of gland infections	Transmissibility index
M 684	Mar. 1930	460	13	2·8	6	11	1·3	*1·5*
M 685	Mar. 1930	395	4	1·0	4	4	1·0	*1·0*
	Totals	855	17	1·9	10	15	1·1	*1·2*

This strain was, therefore, still transmissible up till the death of the patient.

Obviously a strain of trypanosomes cannot experience exactly the same influences in different animals, even if they are of the same species, and it is only to be expected that a comparatively mild parasite like *T. gambiense* will react in different ways in a succession of hosts. The cerebro-spinal fluid cell count is, moreover, a fallible criterion of the length of infection, as the resistance of the individual host plays an important part in determining the progress and duration of the disease, to say nothing of intercurrent ailments. But these considerations are outside the defined scope of this paper and the matter demands much more study.

IV. Discussion.

Reflection on the evolution of those flagellates whose life history includes an alternation of hosts and of generations brings to mind the two main hypotheses that have been advanced to explain the origin of this phenomenon.

First, there is the view originally put forward by Léger, that the lower trypanosomidae were at first parasites of insects in whose intestine all the stages of their life cycle were passed. Passage from insect to insect was effected by contaminated feeding. Eventually some of these insects acquired a blood-sucking habit, and the flagellates, being ingested by the vertebrate, found their way into the blood and tissues and gradually adapted themselves to the new environment. Hoare (1923) developed this view to account for the inoculative method of infection in vogue among the pathogenic mammalian trypanosomes, by supposing that a gradual transformation from mechanical to cyclical transmission took place. In this way a trypanosome which was naturally transmitted through the hind-gut of its intermediate host was taken up from the blood by other biting insects and transmitted mechanically, the trypanosome gradually adapting itself to some mechanical transmitter and undergoing therein a cycle of development culminating in the establishment in the anterior station of metacyclic infecting forms.

The alternative explanation is that the trypanosome was primarily a parasite of the vertebrate and learnt to avail itself of the mobility and voracity of the bloodsucking invertebrate, insect or leech as the case might be.

Within the genus *Trypanosoma* are to be found various degrees of adaptation of the trypanosome to its invertebrate host. The phenomenon before us is the association of certain mammalian trypanosomes with *Glossinae*; and the particular aspect of this phenomenon that for the moment occupies our attention is the obvious imperfection of this association. Compared with *T. melophagium*, *T. lewisii*, the frog and fish trypanosomes, and even such tsetse-carried species as *T. uniforme* and *T. vivax*, *T. brucei* and its allies have but a feeble hold over their intermediate host. Dependant as they are upon the *Glossinae* for their dissemination in nature, the security of these trypanosomes is jeopardised from several quarters. Tsetse are catholic in their tastes and feed readily upon vertebrates in whose blood the trypanosomes cannot survive, for instance reptiles and baboons. This consideration applies with

particular force to *G. palpalis* and other reptile-loving species upon which *T. gambiense* chiefly relies for its propagation. Although the influence of this factor upon the survival of the trypanosome is admittedly indirect, it certainly indicates the importance to the parasite of a ready capacity for establishing itself in the tsetse whenever occasion offers. But here we are confronted with the remarkable fact that, even in the most favourable circumstances, the percentage of tsetse that becomes infected with these polymorphic trypanosomes is very small. Even in the laboratory where, apart from such enervating and artificial stimuli as confinement in an incubator at 37° C., the flies can be offered every opportunity to take up infective trypanosomes, this percentage remains very small in comparison with the standard attained either by the species of trypanosome mentioned above or by other parasitic protozoa in their adaptation to their chosen intermediate host. Thus James has shown that under certain conditions 100 per cent. of anophelines can transmit malaria cyclically; according to the work of Theiler, the species of tick concerned with the transmission of East Coast fever transmits the parasite with great readiness; and the same is true for the species of *Ornithodorus* and *Pediculus* that act as intermediate hosts for the different species of *Treponema*. With *Glossinae*, on the other hand, exposed to trypanosomes of the *brucei* group, 10 per cent. of infected flies is a high figure.

What is the explanation of this inferiority? Does the responsibility rest with the trypanosome or with the fly or with both? And here obtrudes another question. What evolutionary interest has the fly in the development of friendly relations with the *brucei* group of trypanosomes? There is no evidence that the fly benefits in any way by the presence in its interior of hordes of developing flagellates, though we have some reason for believing that it suffers no disability therefrom (Duke, 1928*a*). There is no evidence that the tsetse-carried mammalian trypanosomes originated within recent times from primary tsetse parasites. All the common flagellates hitherto detected in the alimentary canal of *Glossinae* have now been proved to be developmental forms of trypanosomes of vertebrates. So that it is difficult to imagine that the fly is in any way interested, or has anything to gain by extending hospitality to the trypanosome, On the contrary, inasmuch as the trypanosome may injure its mammalian host, the fly, which to a considerable extent depends on those hosts for blood, stands to lose by helping in the spread of the parasite. In other words, there seems to be no biological incentive for selected variation on the part of the fly in a direction for or against co-operation with the trypanosome.

On the other hand, the trypanosome will undoubtedly benefit by improving its relations with the tsetse, and therefore it seems reasonable to assume that it is on the ability of the trypanosome to produce variations suited to the task of invading the fly that the success of this, biologically speaking, new venture depends.

This then is the interpretation which I adopted, as a working hypothesis, as the result of a study of the transmissibility of trypanosomes carried out with the League of Nations Commission in 1926–1927.

On beginning these studies, I assumed that certain of the short forms described by Miss Robertson were alone capable of surviving in tsetse, and that every time such a form was taken up by a fly infection of that fly would ensue. But during the last eighteen months the weight of experimental evidence tends rather to show that in only a comparatively small percentage of tsetse can any development of trypanosomes take place, this percentage varying according to the adaptability possessed by the strain, so that some strains are more transmissible by tsetse than others.

Moreover, it is probable that the hypothetical forms destined for development in the fly must be taken up by the insect in adequate numbers before development can occur, just as malarial gametocytes if present in sufficient numbers do not produce infection of the mosquito, the transmissibility of a strain being thus directly dependent on its output of these particular forms.

To anyone familiar with the nature of such work it will be evident that experimental proof is difficult to obtain, but the view that the trypanosome is the variable factor rather than the fly seems to me to be based on sound reasoning. Miss Robertson, dealing with this same problem, speaks of the "general inhibiting capacity of the *Glossinae*" (Robertson, 1913). I have hitherto regarded this factor as a passive obstacle against which the trypanosome has constantly to contend, and in the early stages of the struggle, which are proceeding before our eyes, its success is at present limited to the small percentage of tsetses in which this "inhibiting capacity" is feebly developed. The task of overcoming the more resistant majority has not yet been accomplished by any of the species of mammalian trypanosomes that depend on tsetse for cyclical transmission. Among these there is a gradation of attainment, with the proboscis-group, *T. vivax* and its allies at one end, and *T. gambiense* at the other. Moreover, it is interesting to note that with the improved adaptation of the species to the tsetse its adjustment to the mammal has also improved, the *vivax* group being almost non-pathogenic, whereas *T. gambiense* is still but ill-adjusted to man.

Nevertheless, while following this line of work, it is necessary to bear in mind the experiments of Huff (1929) with *Culex pipiens* and *P. cathemerium*, and the possibility that variations in the susceptibility of different races of tsetse to infection may play a part in determining the spread of trypanosomes in nature. Experiments are being devised to find out whether *G. palpalis* from different localities differ appreciably in their susceptibility to infection.

That the results set forth above are not entirely or even mainly due to this cause is however quite certain, for other flies hatched from the same consignments of pupae as furnished the experiments described in this paper, when fed concurrently upon transmissible strains of either *T. gambiense* or *T. rhodesiense*, showed a due proportion of gland infections.

As the result of some years of study under conditions of experiment that are remarkably constant, transmissibility by tsetse seems to me to be an

unstable character whose very instability suggests its comparatively recent acquisition.

There are reasons for believing that *T. brucei* has progressed further towards perfect adaptation to both definitive and intermediate hosts than has *T. gambiense*, which is still a serious menace to its principal host, man. And although it seems certain that the same laws will be found to apply to both these trypanosomes, and indeed to all the species that depend upon tsetse, it is probable that the secrets of past progress and the weaknesses of the present position can be better appreciated by studying a trypanosome that is still definitely pathogenic to its principal mammalian host.

If further work shows that strains of the *T. brucei* type of trypanosome from game tsetse regions are consistently more infective to our laboratory bred *G. palpalis* than are strains of *T. gambiense* derived from *G. palpalis* regions, this will be additional evidence that it is indeed the trypanosome rather than the fly upon which the transmissibility of the strain depends.

I have elsewhere described the stages by which transmissibility is lost (Duke, 1923, 1924, 1924*a*); how there is an intermediate stage in the declining career where the power to infect the glands disappears, the strain remaining readily infective to the gut of the fly. At such a stage the strain is of course already non-transmissible. The existence of this stage is, I hope, proved by the evidence in Table II.

Once loss of transmissibility has been proved to take place, there is nothing unreasonable in the claim that there are strains in existence in nature that have retrogressed still further until their infectivity to fly has almost or quite disappeared, although from an epidemiological standpoint the distinction possesses no importance.

As already stated, no attempt is made in this paper to discuss the causation or the constancy of these changes. Its object will be fulfilled if the evidence submitted is deemed adequate to prove the principle that a strain of trypanosomes, originally introduced into a mammal by the bite of a cyclically infected tsetse, may in time in one and the same animal lose completely and permanently the power of cyclically infecting *Glossinae*.

V. CONCLUSIONS.

1. Evidence is submitted that strains of *T. gambiense*, non-transmissible by *G. palpalis*, occur in man in nature in regions where *G. palpalis* is the normal vector of this trypanosome.

2. Such strains may be still infective to tsetse, but cannot invade the salivary glands of the fly.

3. The application of successive tests to one and the same animal supplies further evidence that the infectivity of a strain to fly is liable to diminish as the disease in the animal progresses towards a fatal termination.

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ON THE CORRELATION OF THE LIFE-HISTORY OF THE ACEPHALINE GREGARINE, *GONOSPORA*, WITH THE SEXUAL CYCLE OF ITS HOST.

II. *GONOSPORA* (*KALPIDORHYNCHUS*) *ARENICOLAE*.

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In a previous paper (1926) I pointed out the very close correlation that exists between the life-history of the acephaline gregarine *Gonospora varia* Léger and the sexual cycle of its host *Audouinia* (*Cirratulus*) *tentaculata* (Montagu). I there showed that the development of the parasite proceeded parallel with that of the worm's gametes and that the parasites produced their sporocysts just prior to the time of spawning; and I suggested that both the sporocysts and the gametes were ejected from the worm together. I further suggested that the development of the parasite was possibly dependent on some stimulating substance produced by the worm's gonads.

In view of this, I thought it might be worth while to see if a similar correlation was to be found in any other species of *Gonospora*, and the present paper gives the result of two years' observations made on *Gonospora* (*Kalpidorhynchus*) *arenicolae* (Cunningham)[1] from the coelom of *Arenicola ecaudata* Johnstone.

I was led to make these observations by a statement of Cunningham (1907), which made it probable that in *Gonospora arenicolae* he was dealing with an analogous case to that of the parasite of *Audouinia*. He says "the cysts were very abundant in specimens containing large quantities of generative products, so that there is some reason for concluding that the cysts escape chiefly, if not exclusively, when the worms discharge their ova and spermatozoa." Robinson (1910), however, who worked on the cytological changes in the formation of the gametes, makes no reference to any possible correlation.

I, therefore, thought it might be interesting to make periodic examination of *Arenicola ecaudata*, as I had done in the case of *Audouinia*, to see if a similar correlation exists. I have had *Arenicola* sent to me from Plymouth, to which

[1] I follow Pixell-Goodrich (1916) in including this form in the genus *Gonospora*. I am the more convinced of the correctness of this view owing to the way in which this acephaline resembles other species of *Gonospora* in the correlation of its life-history with the host's sexual cycle. This correlation seems a marked feature of the genus.

I also made two visits for the purpose of examining a larger amount of material. I may here express my thanks to the University of London for the use of their table at the Marine Biological Laboratory and also to the Royal Society for a grant towards the cost of the material sent to me in London. During two years I examined nearly 300 worms. These, however, were not all *A. ecaudata*, but included specimens of *A. grubii* among the worms I examined on my first visit to Plymouth. When I first began this work, I was ignorant of the existence of *A. grubii*, which is not infected by the gregarine. As is pointed out by Gamble and Ashworth (1900), these two species greatly resemble each other in external features and are difficult to distinguish; moreover, confusion between the two is easy, as they share the same habitat.

According to Ashworth (1904), the breeding season of *A. ecaudata* is from the latter part of February to the beginning of March. On the analogy of *Gonospora varia*, I expected to observe large trophozoites in the autumn, and to find association stages and the formation of gametocysts and subsequently of sporocysts taking place in the early part of the year. This assumption did not, however, prove to be entirely correct. The reason for this is that I am now not at all certain that the worm has such a definite annual spawning season. While it is undoubtedly correct that spawning occurs in February and March, to judge from the examination of the genital products it would appear that some of them may breed at various times during the summer months, and it is quite possible that there is a fairly well-marked second spawning period in August.

When I first studied *Arenicola* in the late autumn (October and November) I found that the ova were still contained in the digitiform ovaries (though in the males the spermatozoa were free and in some specimens active). At the same time many trophozoites of *Gonospora arenicolae* were attached in clusters to the nephridial funnels, as described by Cunningham (1907). This is in sharp contrast with the corresponding stage in *Audouinia* where both gametes and parasites are shed free into the coelom very early. It was not until December that I first came across cysts, and it was noticeable that these were, with one exception, in worms in which the ova had been shed free in the coelom from the ovaries. (The one exception was a worm in which the whole body cavity and the ovary seemed full of trophozoites and cysts, though the ova were still in the ovary. I shall offer a possible explanation of this anomaly later.)

From this time onwards worms with gametes free in the coelom became gradually commoner, and the number of cysts increased in marked correlation. This state of affairs seemed to reach its climax about the middle of February, which is probably the beginning of the breeding season. The cysts about that time mostly contained mature sporocysts. Thereafter worms were found with little or no genital products in the coelom, and the gonads were generally small and poorly developed. This would indicate that the worms were spent by the latter part of March, and I, therefore, expected to find little trace of genital products in the worms during the summer, and only the youngest stages of the

gregarine. This, however, proved not to be the case, for during the summer months worms were found which not only contained ova and spermatozoa in various stages of maturity, but also well-developed trophozoites and, in the most mature worms, gametocysts. Furthermore, such animals seemed to become more numerous as the summer progressed, until by August most of the worms seemed again to be in a condition just prior to spawning.

It would therefore seem that *A. ecaudata* has two spawning periods each year, one in February–March, and the second August–September. On the analogy of *Audouinia* there should be two complete cycles in the life-history of the parasite, but this does not appear to be so. In *Audouinia* the worms seem to rid themselves not only of all their genital products but also of all the sporocysts of *Gonospora varia*; and reinfection has to occur by the entry of sporocysts into the worm, presumably through the mouth. Thus only young forms of the parasite are found in the autumn, for the old unassociated trophozoites seem to degenerate during the late summer months. In *Arenicola*, on the other hand, the correlation between the life-history of the gregarine and the sexual cycle of the host is not nearly so clear cut. In nearly all, if not all, infected worms, there always seem to be a few trophozoites still attached to the nephridia, even when the body cavity is full of gametes and cysts. These do not appear to degenerate after the worm is spent, but remain to associate and form sporocysts for a later spawning period.

In my previous paper I suggested that the impulse which stimulates the gregarines to their reproductive processes, might be due to some secretion produced by the worm's gonads. This may also be true of *Gonospora arenicolae*, since associated forms and cysts were nearly always found only where the gametes were lying free in the coelom. This feature is particularly noticeable in female worms (which incidentally are much more frequently and more heavily infected than the males), because the ova are shed into the coelom at a much later stage than are the spermatozoa. When the gonads had not burst, it was the general rule to find only unassociated trophozoites attached to the nephridial funnels. It would seem, therefore, that the secretion does not diffuse out into the coelom and is only liberated on the bursting of the ovaries. Until this happens, the trophozoites remain attached without developing further. Confirmation of this hypothesis may possibly be obtained from the behaviour of the one exceptional specimen, already mentioned, in which an abundance of cysts was found without liberation of the ova. At first sight this would seem to contradict the theory, but possibly it may be explained by the fact that the parasites had in this specimen penetrated the ovaries and in so doing may have caused the liberation of the secretion, which had therefore stimulated the gregarines to associate.

In my study of the gregarines of *Audouinia*, I was unable to complete the chain of observations on the two correlated cycles, but was compelled to assume that the sporocysts were shed into the surrounding water at the same time as the worm's gametes. Unfortunately *Audouinia* spawns in May, when it was

impossible, being term time, for me to be at Plymouth. I endeavoured to keep the animals in aerated water in London, but owing to the warm weather at that time of the year and the decaying mud in which the worms were entangled, the water always rapidly became foul and the worms died. With *Arenicola* I was more successful. If a steady stream of air was kept bubbling through the water, these worms lived quite well in glass jars containing a number of small pebbles among which they could hide. As the time of the year was February to March, the water did not get very hot and so did not foul in a few hours. The chief difficulty in keeping the worms any length of time is the problem of feeding them; for it is impossible to permit the presence of much detritus on which they live, for fear of polluting the water. If they are left in the water without any food they will live for a considerable time, but, although distended with gametes, will not spawn. It was found in two such specimens, which were kept alive for several weeks, that owing to starvation the worms were probably living at the expense of their own ova, which were vacuolated and showed conspicuous signs of degeneration.

Success, however, was achieved in a number of cases when the worms spawned within a few days of being placed in the tank. If they did not do so within a week, they were not allowed to remain longer. It may be of interest briefly to put on record the behaviour of the worms in the tank. They soon hid themselves as best they could among the pebbles and proceeded to make a series of membranes of a very thin, transparent, slimy material, which they secreted themselves and attached to the pebbles. Beneath this sort of awning the worms occasionally moved about from place to place, though they were usually fairly inert. When they were spawning they were more active, travelling slowly about among the stones and, in so doing, pouring out their genital products, most of which were caught among the covering membranes. This may possibly be the method adopted under natural conditions, for these worms live ordinarily on ground where there is a mixture of pebbles and sand (not in sand alone like *A. marina*). Though they tend to embed themselves fairly deeply, they probably come nearer to the surface to breed. (An attempt at keeping them in the laboratory in a tank containing some coarse gravel was not at all successful, as they promptly burrowed into it and were seen no more till they died of starvation.)

Of the specimens which spawned in the tank, it was unfortunate that all except one were so slightly infected with *Gonospora* that I could not see whether the sporocysts of the gregarine were ejected together with the genital products. One worm, however, started to spawn vigorously almost immediately after it was placed in the tank. The matter ejected was pipetted off and examined, and was found to consist of both ova and sporocysts. It would appear, therefore, that the assumption that this is the method by which the sporocysts are disseminated is correct. The method of spawning within a confined space would render infection easy, especially as the membranes are not used by any particular worm but are the common property of all.

There is one problem which still remains. How do the gametocysts burst and so liberate the sporocysts into the coelom prior to their ejection to the exterior? The presence of "phagocytes" is a very striking feature in *Arenicola* in contradistinction to *Audouinia*, where they appear to be absent or very few in number. These cells become much more numerous when the gametes are shed free into the coelom, and remain in enormous quantities for some time after the worm is spent. They do not cluster round the unassociated, naked trophozoites, but gather in great masses about the gametocysts. It may be tentatively suggested that the action of the phagocytes may eventually cause a rupturing of the gametocyst wall, which in *G. arenicolae* is rather thin, as contrasted with the tough, opaque wall of *G. varia*. In this way the sporocysts may be liberated into the coelom.

SUMMARY.

1. In general there is a correlation between the life-history of *Gonospora arenicolae* and the sexual cycle of its host, *Arenicola ecaudata*, similar to that between *G. varia* and *Audouinia tentaculata*.

2. In the species under consideration the correlation is not so definite and the life-history of a generation of parasites does not necessarily coincide with a sexual cycle of the host. This is complicated by the double annual spawning of the host.

3. The formation of sporocysts is simultaneous with the sexual maturity of the worm.

4. It is suggested that, as in *Audouinia*, a secretion produced by the gonads stimulates the development of the gregarine.

5. The ejection of the sporocysts with the gametes was observed.

6. It is suggested that phagocytosis may be the cause of the rupture of the gametocysts in this species.

In conclusion, I would express my thanks to Prof. Doris L. Mackinnon, of King's College, London, for her kind help and criticism.

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